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RESEARCH ARTICLE

Population genetic structure and colony breeding system in dampwood termites (*Zootermopsis angusticollis* and *Z. nevadensis nuttingi*)

W. Booth · C. S. Brent · D. V. Calleri · R. B. Rosengaus · J. F. A. Traniello · E. L. Vargo

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Abstract Studies describing the population genetic structure and breeding system of basal lineages of termite species remain rare. Such species, however, may reveal ancestral life history attributes potentially influential in the evolution of social life within the Isoptera. Through the development and application of microsatellite DNA loci, we investigated patterns of genetic diversity and differentiation within the dampwood termite *Zootermopsis angusticollis* collected from three geographically distinct locations in California, USA. Significant genetic differentiation was identified among all sites, which were located 40–150 km apart, and each site was found to represent unique populations with limited levels of gene flow. While *Z. angusticollis* alates have previously been described as being strong fliers, genetic evidence suggests limited dispersal, possibly due to habitat characteristics restricting long-range flights. Additionally, we characterize patterns of colony genetic structure and breeding system within both *Z. angusticollis* and its congener *Z. nevadensis nuttingi*. In *Z. angusticollis,* simple, extended, and mixed family colonies were observed. The frequency of simple families ranged from 16 to 64%, whereas mixed families were found in only two locations and at low frequencies. In contrast, *Z. n. nuttingi,* formed primarily extended family colonies. Estimates of relatedness suggest that monogamous pairs heading simple families consist of reproductives showing variable degrees of relatedness from unrelated to close relatives. Additionally, the effective number of neotenic reproductives appears to be low within extended families of both species.

Keywords Ancestral termites · Termopsidae · Isoptera · Gene flow · Breeding system · Microsatellites · COI

W. Booth (✉) · E. L. Vargo
Department of Entomology and W. M. Keck Center for Behavioral Biology, North Carolina State University, Box 7613, Raleigh, NC 27695, USA
e-mail: warren_booth@ncsu.edu

E. L. Vargo
e-mail: ed_vargo@ncsu.edu

C. S. Brent
USDA-ARS, Arid-Land Agricultural Research Center, 21881 North Cardon Lane, Maricopa, AZ 85138, USA
e-mail: Colin.Brent@ars.usda.gov

D. V. Calleri
University of Chicago Laboratory Schools, 1362 E. 59th St., Chicago, IL 60637, USA
e-mail: dcaller@ucls.uchicago.edu

R. B. Rosengaus
Department of Biology, Northeastern University, 134 Mugar Life Sciences, 360 Huntington Ave., Boston, MA 02115, USA
e-mail: r.rosengaus@neu.edu

J. F. A. Traniello
Department of Biology, Boston University, 5 Cummington Street, Boston, MA 02215, USA
e-mail: jft@bu.edu

Introduction

Genetic studies comparing breeding systems and population genetic structures of social insects have provided important insights into the diversity of their patterns of social organization, evolutionary genetics, and maintenance of eusociality. However, such studies have largely focused on the eusocial haplodiploid Hymenoptera (Crozier, 1977; Cole, 1983; Pamilo et al., 1997; Ross, 2001; Wilson and Hölldobler, 2005; Hughes et al., 2008; Menke et al., 2010). In recent years, there has been an increasing number of studies on the

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diploid termites (Isoptera), greatly expanding our understanding of colony breeding structure and dynamics in eusocial species (reviewed in Vargo and Husseneder, 2009, 2011).

The vast majority of studies on colony and population genetics of termites have concerned derived lineages, especially subterranean species (Vargo and Husseneder, 2009, 2011), illustrating great variation in colony breeding structure within and among species. Based on the number and developmental origin of reproductives present, termites can be classified into colonies headed by: (1) a single pair of monogamous reproductives (simple families); (2) multiple inbreeding neotenics (extended families); and (3) multiple unrelated reproductives (mixed families). Most populations of subterranean termites consist of varying proportions of simple and extended families, whereas mixed families tend to be less common, except in some introduced populations (e.g., Perdereau et al., 2010). Consequently, levels of inbreeding vary considerably among species and even among populations within a species (Vargo and Husseneder, 2009, 2011), although in many cases inbreeding levels are low.

Beyond illuminating variation at the colony level, the determination of genetic differentiation and the estimation of patterns of gene flow within and among samples collected in geographically distinct regions or localities provide valuable information regarding a species' population dynamics and dispersal patterns (Avise, 2004). This information can help elucidate past and present ecological factors, such as habitat fragmentation and barriers to gene flow, that may significantly influence the level of genetic diversity and its partitioning among populations (Goodisman and Crozier, 2002; Lefebvre et al., 2008; Booth et al., 2009, 2011; Crissman et al., 2010). Of the few studies of this type conducted in termites, the majority have concentrated on derived lineages such as Rhinotermitidae, many of which are considered pest species with extensive geographic ranges. Genetic differentiation and gene flow in termite species that are habitat specialists are relatively unexplored. Additionally, there are few studies on the colony genetic structure of basal termites. The most basal family, Mastotermitidae, is represented by a single species, *Mastotermes darwiniensis*. In the sole genetic study of the colony and population genetic structure of this species, Goodisman and Crozier (2002) found that approximately half of the colonies they studied (9 of 19) were extended families, whereas simple and mixed families each comprised about one-quarter of the colonies.

The Termopsidae, another basal termite group (Inward et al., 2007) exhibits the most ancestral characteristics in terms of its life history traits and social organization (Thorne, 1997). In North America, this family is represented by *Zootermopsis*, a genus consisting of three species: *Z. angusticollis* (Hagen), *Z. laticeps* (Banks), and *Z. nevadensis* (Hagen). The latter is subdivided into the subspecies *Z. n. nevadensis* and *Z. nevadensis nuttingi* (Haverty and Thorne, 1989; Thorne et al., 1993). *Z. angusticollis* and *Z. nevadensis* occur along the Pacific Coast, whereas *Z. laticeps* is found in the southwestern US. *Zootermopsis* spp. are habitat specialists, exhibiting single-piece nesting, generally within the moist, decaying wood of rotting logs and wood debris (Thorne et al., 1993; Rosengaus et al., 2003). Unlike more derived termite lineages, a true worker caste (i.e., an irreversible line of non-soldier, non-reproducing individuals) is absent and instead all non-reproductive individuals can differentiate into any other caste (i.e., referred to as pseudergates) (Thorne, 1997). Although many aspects of their biology and biogeography have been well studied (Haverty and Thorne, 1989; Thorne et al., 1993; Thorne, 1997; Brent et al., 2008; Johns et al., 2009), little is understood of their colony and population genetic structure (Broughton, 1995; Shellman-Reeve, 2001; Aldrich, 2005; Aldrich and Kambhampati, 2007, 2009), and nearly all research has concerned only a single species, *Z. nevadensis*.

In this paper, through the development and application of high resolution microsatellite markers, we elucidate population genetic structure and genetic differentiation within and among three populations of *Z. angusticollis* collected in California, USA. We then provide a genetic analysis of the colony structure and breeding system of *Z. angusticollis* and compare it to a population of its congener, *Z. n. nuttingi*.

Materials and methods

Sample collection and DNA extraction

Samples were collected from wood debris (tree stumps and downed decayed logs) between late October 2002 and early September 2003 at three locations within California: Pebble Beach, ($n = 11$) Redwood Regional Park, Oakland ($n = 19$), and Huddart County Park, Woodside ($n = 22$) (Fig. 1). Within each sample site, a minimum of 10 pseudergates were collected from each colony and preserved immediately in 95% ethanol. Samples were then stored at −20°C prior to DNA extraction. Total genomic DNA was extracted from 10 to 20 pseudergates from each colony using the PUREGENE DNA isolation kit (Gentra Systems Inc., Minneapolis, MN, USA).

Mitochondrial DNA sequencing and species determination

Species and subspecies determination within this genus has been successfully achieved through both hydrocarbon analysis (Haverty and Thorne, 1989) and molecular

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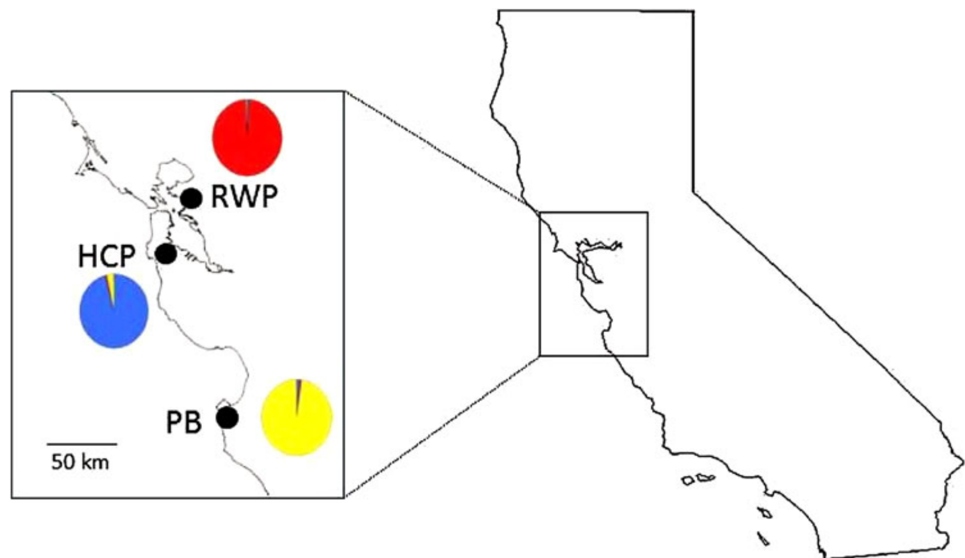

Fig. 1 Collection locations within California, USA. *Colored pies* represent the proportion membership of each of three population samples (*HCP* Huddart County Park, *PB* Pebble Beach, *RWP* Redwood Regional Park) to three genetic groups identified following Bayesian STRUCTURE analysis. Results represent five sequential runs aligned using CLUMPP

approaches (Aldrich and Kambhampati, 2009). To accurately identify species, cytochrome oxidase I (COI) gene sequences, commonly used in ''DNA barcoding,'' were amplified using primers LepF1 (5′-TTCAACCAATCAT AAAGATATTGG-3′) and LepR1 (5′-TAAACTTCTGGA TGTCCAAAAAATCA-3′) (Hebert et al., 2004; Hajibabaei et al., 2006). PCR was performed in 30 µl volumes containing: 1X PCR buffer, 2 mM $MgCl_2$, 100 mM dNTPs, 0.3 µM of each primer, 0.5 U Taq DNA polymerase (Bioline, Taunton, MA, USA), ~50 ng DNA template, and ddH_2O to 30 µl. PCR cycling conditions were comprised of an initial denaturation stage of 1 min at 94°C, followed by four cycles each consisting of 1 min at 94°C, 1.5 min at 45°C, and 1.5 min at 72°C. This was followed by 34 cycles each consisting of 1 min at 94°C, 1.5 min at 45°C, and 1 min at 72°C. All 56 DNA extracts amplified under these conditions. PCR products were visualized on a 2.5% agarose gel to confirm that samples contained only a single band. 5 µl of PCR product was subsequently purified using the ExoSAP-IT PCR purification kit (USB Corporation, OH, USA) and bidirectionally sequenced following the methodology outlined in Copren et al. (2005). Sequence alignments were performed using the Vector NTI Advance 10 program (Invitrogen, Carlsbad, CA, USA). Phylogenetic relationships were examined using Molecular Evolutionary Genetics Analysis (MEGA), version 4 (Tamura et al., 2007). Neighbor-joining (NJ) analysis was performed with all characters weighted equally and bootstrap replicates set to 10,000.

Microsatellite screening

Samples were screened at nine microsatellite loci. Four (*Zoot31, Zoot73*, *Zoot101*, *Zoot117*) were previously described by Aldrich and Kambhampati (2004) and five (*Za-18*, *Za-123*, *Za-127*, *Za-132*, *Za-197*) were developed here for this species following the procedures of Vargo and Henderson (2000) (Table 1). Loci were multiplexed into two sets (Set 1: *Za-127*, *Za-132*, *Za-18*, *Za-197*; Set 2: *Za-123*, *Zoot117*, *Zoot101*, *Zoot73*, *Zoot31*). PCR conditions for *Zoot31, Zoot73*, *Zoot101*, *Zoot117* followed those outlined by Aldrich and Kambhampati (2004), while those for the loci developed here are outlined in Table 1. Amplified products were labeled with M13F-29 IRDye tags (Li-Cor Biosciences, Lincoln, NE, USA). Polymerase chain reaction (PCR) products were separated by electrophoresis on 6% polyacrylamide gels run on a LI-COR 4300 dual-laser automated sequencer. Loci were sized using a 50–350-bp standard (Li-Cor™) run for every 15 samples. At least one control sample (i.e., a sample of known genotype) was included in each run to ensure accuracy and consistency of scoring among different gels. The GeneProfiler (v4.05) software (Scanalytics, Rockville, MD, USA) was used to collect genotypic data from the LI-COR system.

Microsatellite data analysis

MICRO-CHECKER v2.2.3 software (Van Oosterhout et al., 2004) was used to assess the likelihood that null alleles, scoring error, or large allele drop out were evident at all loci screened. Summary population statistics (allelic diversity, expected and observed heterozygosity) were calculated using the Genetic Data Analysis (GDA) v.1.1 software (Lewis and Zaykin, 2001). Tests for departures from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were calculated using GENEPOP v4.0 (Raymond and Rousset, 1995; Rousset, 2008). The Markov Chain parameters were set to 2,000 dememorisations, 200 batches and 2,000 iterations per batch. Given the non-independence of genotypes among individuals within a colony resulting from

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Table 1 Characteristics of five microsatellite DNA loci developed for the dampwood termites *Zootermopsis angusticollis* and *Z. nevadensis nuttingi* screened for a total of 963 and 260 specimens respectively

Locus	Primer sequences	Motif	Annealing temperature (°C)	No. of cycles	Primer Conc (μM)	mM $MgCl_2$	Expected product size	N_A		Genbank accession no.
								Z. angusticollis	*Z. n. nuttingi*	
Za-18	F: TGATTGGTTGGTCGTTCAGA R: TGCGTTATTGCGTCGTTAAA	$(CA)_{10}$	54	28	1	2	245	6	4	JN186786
Za-123	F: CGTATTCCTGCCCCTTCATA R: CCGGATATACGGCAAACACT	$(GT)_{13}$	54	28	1.25	1.75	106	7	3	JN186787
Za-127	F: GCAGTAGCATCTCCATTCCG R: GAGGAAGAGATCGTATGTGTTGAA	$(CA)_{12}$	54	28	1	2	102	7	3	JN186788
Za-132	F: GCTGAAATGGCTCGTGAAAT R: CGTCAGCCTCGTTTAAAGCAT	$(AC)_{14}$	54	28	1.5	2	179	1	3	JN186789
Za-197	F: TCACGCACCATTTCTCTGAC R: GCCTTGTCCACCAGAATCTC	$(TC)_{10}$	54	28	0.8	2	292	25	15	JN186790

N_A Number of detected alleles

familial relatedness, a single individual was chosen at random for each analysis. Each species was analyzed separately with 10 replicates for each analysis.

Population genetic structure

Evidence for departures from panmixia was assessed among samples from discrete sites using a number of independent approaches. Pairwise genotypic differentiation was tested using the log-likelihood based *G* test (Goudet et al., 1996), implemented in GENEPOP. Departures from panmixia were used to group collections into distinct colonies following the method proposed by Waples and Gaggiotti (2006), whereby samples are considered to be part of the same colony if they could be connected to other samples by a non-significant *G* test. Genetic differentiation, both overall and between pairs of sampled locations, was also determined by means of Weir and Cockerham's (1984) estimator θ of F_{ST}, as implemented in the program FSTAT (Goudet, 2001). The Bayesian clustering algorithm implemented in STRUCTURE v2.2.3 (Pritchard et al., 2000) was applied as an exploratory analysis to determine whether the colony samples could be subdivided into *K* clusters (where *K* is unknown), each characterized by a set of allele frequencies at each locus. Under this method, individuals are probabilistically assigned to each cluster based on the proportion of their genome that matches that cluster. Runs were based on 250,000 iterations following a 50,000 burn-in period. Runs were replicated a total of five times to check concordance of the data with *K* set between one and five. STRUCTURE analysis was performed assuming the admixture model with allele frequencies correlated. Given the presence of known families within the data set, analysis was performed on a single individual chosen at random from each colony. The optimal value of *K* was identified following the ΔK method described by Evanno et al. (2005) using the STRUCTURE HARVESTER v0.6 software (Earl, 2011). Replicated STRUCTURE runs were aligned using the program CLUMPP (Jakobsson and Rosenberg, 2007) to maximize each individual's membership across clusters.

To test for genetic isolation by distance, regression analysis of pairwise F_{ST} values and geographic distances was performed using Mantel's randomization test (Mantel, 1967). Geographic distances were log transformed and genetic distances transformed to $F_{ST}/1\text{-}F_{ST}$. Analysis was performed using MANTEL v2 (Liedloff, 1999) employing a total of 10,000 permutations.

Following a recent, severe reduction in a population's effective size (i.e., a genetic bottleneck), a characteristic genetic signature of an excess in heterozygosity may be observed at selectively neutral genetic markers, such as microsatellite loci. This occurs as allelic richness declines faster than heterozygosity due to the loss of rare alleles that

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contribute little to overall heterozygosity (Cornuet and Luikart, 1996). To determine if a recent bottleneck had occurred, the distribution of allelic frequencies was analyzed following the Wilcoxon statistical test, and the qualitative graphical method (i.e., mode-shift) suggested by Luikart et al. (1998), which are implemented in the program BOTTLENECK v1.2.01 (Cornuet and Luikart, 1996). The Wilcoxon test was chosen as it is considered robust when using fewer than 20 unlinked microsatellite loci (Piry et al., 1999). As microsatellite loci are unlikely to strictly follow the stepwise mutation model, analysis was run assuming two alternative mutation models: the Infinite Allele Model (IAM), and the Two-Phase Model (TPM). The latter was run assuming 95% single-step mutations.

Colony genetic structure and breeding system

Colony breeding structure was classified following the model of Vargo et al. (2003) and DeHeer and Vargo (2004). Under this classification, colonies are categorized into one of three groups; simple, extended or mixed families. Within simple families, the genotypes of workers follow those expected for a single pair of primary reproductive parents, having a maximum of four alleles and four genotypic classes in ratios expected under simple Mendelian patterns of inheritance. The significance of these ratios is determined through a G test performed per each locus then summed across all loci. Extended families, like simple families, will have no more than four alleles per locus; however, the genotypes of the workers are consistent with multiple reproductives, as identified by the presence of more than four genotypic classes at one or more loci, or by G test values deviating significantly from those expected under a simple family. Extended families can be headed by a mixture of primary and secondary reproductives. Mixed family colonies result from the fusion of two or more colonies with unrelated reproductives and will be evident by greater than four alleles at one or more loci.

Colony genetic structure was investigated using F-statistics following the method of Weir and Cockerham (1984) implemented in the program FSTAT (Goudet, 2001). Analysis was performed by species and by sample location in order that F values used to infer breeding system were not confounded by higher-level genetic structure. The 95% confidence intervals were estimated by bootstrapping over loci with 1,000 replications. Those values that did not overlap zero were considered to be significant at the $\alpha = 0.05$ level. The notation of Thorne et al. (1999) and Bulmer et al. (2001) was followed in which each individual colony is considered as a sub-population and the genetic variation is partitioned among the following components: the individual (I), the colony (C), and the total (T). F_{IT} is analogous to the inbreeding coefficient and is a measure of the level of inbreeding in individuals relative to the population. F_{CT} is comparable to F_{ST} and represents differentiation between colonies. Finally F_{IC} is the coefficient of inbreeding in individuals relative to their colony and is particularly sensitive to the numbers of reproductives present and their mating patterns within colonies. Strongly negative F_{IC} values are expected in simple families; values should approach zero with increasing numbers of reproductives within colonies and be positive with assortative mating among multiple groups of reproductives within colonies or with mixing of adults from different colonies.

For simple families, we inferred the genotypes of the two reproductives heading each colony from the genotypes of the workers. We then estimated the coefficient of relatedness between the two nestmate reproductives using the program Relatedness v.5.0.8 (Queller and Goodnight, 1989).

Results

Mitochondrial DNA species determination

A 612-bp fragment of the COI region was amplified and sequenced for 67 individuals (one individual per colony) to genetically determine species. Of the 612 nucleotide sites, 87 were variable and 69 parsimony informative, resulting in five unique haplotypes split into two distinct species clades. Haplotypes I through III formed one clade and were found to represent *Z. angusticollis*, whereas haplotypes IV and V formed the second clade and represented *Z. n. nuttingi*. Genbank accession numbers are: Haplotype I: JN186791; II: 186792; III: JN186793; IV: JN186794; V: JN186795. Kimura-2 parameter pairwise distances calculated within species ranged from 0.14 to 1.4%, while between species clades distances ranged from 9.31 to 10.09%. All colonies collected at Redwood Regional Park and Huddart County Park were *Z. angusticollis*. Both species were present in Pebble Beach.

Microsatellite summary statistics

Z. angusticollis

Unambiguous genotypes were determined for 963 individuals, with an average of 17.83 individuals per colony (range 8.22–20). Between one (*Za-132* and *Zoot31*) and 25 (*Za-197*) alleles were detected per locus with a mean of 6.33 per locus. Within each sampled location, average expected heterozygosity was 0.306 and average observed heterozygosity was 0.258 (Table 2). The inbreeding coefficient ranged from 0.105 to 0.279 (average 0.163) (Table 2). When pooled, samples were found to diverge significantly

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Table 2 Summary statistics for *Zootermopsis* spp. population samples collected from three locations in California, USA, screened at nine microsatellite loci

	Z. angusticollis					*Z. n. nuttingi*				
Locus	N	N_A	H_E	H_O	F_{IS}	N	N_A	H_E	H_O	F_{IS}
Pebble Beach	11	2.66	0.248	0.182	0.279	15	3.67	0.395	0.260	0.29
Redwood Regional Park	19	2.66	0.335	0.29	0.137	–	–	–	–	–
Huddart County Park	22	3.11	0.335	0.301	0.105	–	–	–	–	–
Mean	17	2.81	0.306	0.258	0.163	15	3.67	0.395	0.260	0.29

Per population: *N* number of colonies screened per location, N_A average number of alleles per locus, H_E expected heterozygosity, H_O observed heterozygosity, F_{IS} inbreeding coefficient, *Mean* overall mean

from Hardy–Weinberg equilibrium. When analyzed by sample location, only the Pebble Beach sample was found to diverge significantly from Hardy–Weinberg expectations following Bonferroni correction. This resulted from a deficit of heterozygotes at locus *Za-197*, and monomorphism at loci *Za-132*, *Zoot31*, *Zoot73*, *Zoot101*, and *Zoot117*. No evidence for linkage disequilibrium or null alleles was detected. Within each population, no evidence of a recent genetic bottleneck was detected.

Z. n. nuttingi

We genotyped 260 individuals with an average of 17.37 individuals per colony (range 9.77–19.77). Allelic diversity ranged from two (*Zoot117*) to 15 (*Za-197*) alleles per locus, with an average of 3.67. Within colony average expected heterozygosity was 0.395, and average observed heterozygosity estimated at 0.260. The inbreeding coefficient was estimated as 0.29. Samples diverged significantly from Hardy–Weinberg equilibrium resulting from a heterozygote deficit at loci *Za-132*, *Za-18*, *Za-197*, *Zoot101*, *Zoot31*, *Zoot73* and *Zoot117*. No evidence for linkage disequilibrium, null alleles, or a recent genetic bottleneck was detected.

Population structure of *Z. angusticollis*

Pairwise genotypic differentiation, as determined by the log-likelihood based *G* test (Goudet et al., 1996), revealed all collection points in both Pebble Beach and Redwood Regional Park populations represented unique colonies. In contrast, at Huddart County Park, three collection points represented a single colony, collapsing the total number of unique colonies within that location to 22. Among the three sample sites, overall F_{ST} was estimated as 0.434 (95% confidence interval: lower 0.296, upper 0.583), whereas between pairs of sites values ranged from 0.269, between Pebble Beach and Huddart County Park, to 0.490 between Pebble Beach and Redwood Regional Park. Results from Structure analysis support the high level of F_{ST} observed between sites with the peak distribution of ΔK observed at 3 (value = 481.50) (Fig. 1). No evidence supported a pattern of isolation by distance ($r^2 = 0.002$, $P = 0.971$, Mantel test).

Comparative colony genetic structure and breeding system

There was considerable variation in the family structure among the three populations of *Z. angusticollis* (Table 3). Simple families and extended families were the only category types in the Pebble Beach population, with the former being the most common. Extended families predominated in the Redwood Regional Park and Huddart County Park populations, and each of these populations had a small percentage of mixed family colonies (Redwood Regional Park = 5.26%; Huddart County Park = 9.09%). The *Z. n nuttingi* population was comprised of two-thirds extended families and one-third simple families.

By definition, the simple family colonies in each population were headed by a single pair of reproductives. Although the simple families in all four populations had positive F_{IT} values (range = 0.133–0.224), none diverged significantly from zero, suggesting that on average the colonies were headed by unrelated primary reproductives (Thorne et al., 1999). This lack of significance is no doubt due to the large variation in the coefficients of relatedness between the reproductives within these colonies. Relatedness values for the monogamous pairs within simple family colonies varied from essentially zero to nearly one across all populations (Fig. 2), indicating that the king and queen in some colonies were unrelated, whereas in others, they were highly related.

As expected, extended families in all populations were significantly inbred. F_{IT} values, the coefficients of inbreeding in individuals relative to their populations, ranged from 0.151 to 0.245 (all $P < 0.03$, one-sample *t* test). However, these values were not as high as those expected after three or more generations of inbreeding ($F_{IT} \geq 0.30$; Thorne et al., 1999), suggesting the colonies had not inbred for more than a generation or two. The strongly negative coefficient of inbreeding in individuals relative to their nest mates for all four populations (all $F_{IC} = -0.195$ to -0.121, $P < 0.02$

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Table 3 Comparative breeding structure and colony genetics of three populations of *Z. angusticollis* and one population of *Z. n. nuttingi*

	F_{IT}	F_{CT}	F_{IC}	r
Z. angusticollis				
Pebble Beach				
All colonies ($n = 11$)	0.156 (0.046)	0.304 (0.029)	−0.212 (0.045)	0.526 (0.038)
Simple family colonies ($n = 7$)	0.133 (0.114)	0.292 (0.065)	−0.227 (0.063)	0.521 (0.071)
Extended family colonies ($n = 4$)	0.185 (0.042)	0.319 (0.028)	−0.195 (0.074)	0.537 (0.049)
Redwood Regional Park				
All colonies ($n = 19$)	0.155 (0.067)	0.246 (0.04)	−0.121 (0.071)	0.43 (0.057)
Simple family colonies ($n = 3$)	0.193 (0.138)	0.278 (0.086)	−0.115 (0.16)	0.469 (0.13)
Extended family colonies ($n = 15$)	0.158 (0.058)	0.253 (0.037)	−0.127 (0.066)	0.438 (0.055)
Huddart County Park				
All colonies ($n = 22$)	0.243 (0.071)	0.335 (0.066)	−0.138 (0.017)	0.544 (0.078)
Simple family colonies ($n = 5$)	0.224 (0.144)	0.395 (0.109)	−0.283 (0.067)	0.657 (0.104)
Extended family colonies ($n = 15$)	0.245 (0.067)	0.339 (0.075)	−0.138 (0.036)	0.549 (0.095)
Mixed family colonies ($n = 2$)	0.333 (0.116)	0.324 (0.068)	0.009 (0.067)	0.492 (0.084)
Z. n. nuttingi				
All colonies ($n = 15$)	0.166 (0.063)	0.298 (0.034)	−0.189 (0.051)	0.512 (0.039)
Simple family colonies ($n = 5$)	0.197 (0.112)	0.390 (0.08)	−0.315 (0.073)	0.657 (0.083)
Extended family colonies ($n = 10$)	0.151 (0.03)	0.243 (0.01)	−0.121 (0.04)	0.422 (0.019)

F_{IT} Standard inbreeding coefficient, F_{CT} genetic differentiation among colonies, F_{IC} colony inbreeding coefficient, r relatedness coefficient

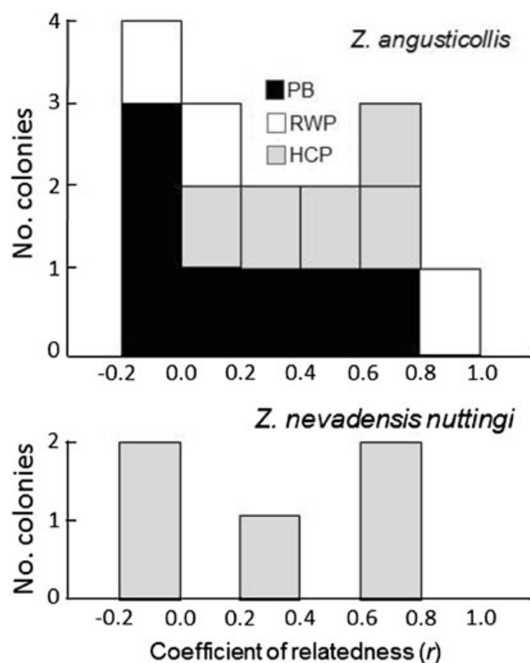

Fig. 2 Histograms of coefficients of relatedness (r) between the kings and queens heading simple family colonies in three populations of *Z. angusticollis* (*HCP* Huddart County Park, *PB* Pebble Beach, *RWP* Redwood Regional Park) and one population of *Z. nevadensis nuttingi* (PB) from northern California. Relatedness estimates are based on inferred genotypes of the reproductives based on the actual worker genotypes present in simple family colonies

for the *Z. angusticollis* Pebble Beach and Redwood Park populations and the *Z. n. nuttingi* population; $P = 0.056$ for the *Z. angusticollis* Huddart County Park population; one-sample t test) indicates a relatively small number of reproductives in these colonies, on the order of fewer than six (Thorne et al., 1999). F_{IC} values close to zero would be expected for 10 or more neotenics.

Discussion

Our results provide the first comparative data on the breeding structure of different species of *Zootermopsis*, an important basal group of termites. These findings build on the results of Aldrich and Kambhampati (2007), validating that sequence variation within the mitochondrial COI gene provides a reliable marker for subspecies determination within *Z. nevadensis* complex through the identification of additional haplotypes within *Z. n. nuttingi*, with sequence divergence estimates between haplotypes within this study ranging from 0.14 to 1.4%. Additionally, we provide evidence for sequence variation between *Z. angusticollis* and *Z. n. nuttingi* comparable to values observed at the interspecific level in other invertebrate systems (Wiemers and Fiedler, 2007), demonstrating that mtDNA sequence variation at the COI gene is a powerful tool for species and subspecies determination in *Zootermopsis* termites.

Population differentiation

We found strong genetic differentiation ($F_{ST} = 0.269$–0.490) over distances of as little as 40 km (see Fig. 1), suggesting limited gene flow at this spatial scale. This is somewhat surprising given that alates of *Z. angusticollis*

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have been reported to be strong flyers, covering distances up to 350 m (Castle, 1934); thus we would expect lower levels of genetic subdivision. However, it is possible that dispersal is limited in this species, either because most alates fly only short distances or because most colonies reproduce by budding (colonies split into two or more fragments and then generate new reproductives from the existing worker force). Although budding has been considered a common mode of reproduction in termites, especially subterranean species (e.g., Thorne et al., 1999), genetic evidence suggests that it occurs infrequently (Vargo and Husseneder, 2011). Moreover, it seems unlikely that budding frequently occurs in dampwood termites because members of a colony do not forage outside of the nest in a stump or log on which they also feed (Abe, 1987). Therefore, the strong population differentiation we observed in *Z. angusticollis* is most likely due to restricted dispersal of alates, possibly resulting from habitat fragmentation.

Our study involved sites fragmented both by urban development and natural barriers. Between Huddart County Park and Redwood Regional Park sites separated by a straight-line distance of only 37.9 km, the high F_{ST} value observed (0.441) may result from the inability of alates to disperse across two major urban areas (San Mateo and Oakland) and the San Francisco Bay, itself covering a distance greater than 20 km. Between the Huddart County Park and Pebble Beach sites, fewer barriers to dispersal and greater habitat connectivity are likely to exist despite being separated by a greater distance, accounting for the lower F_{ST} of 0.269. Finally, the highest level of geographic separation and genetic differentiation ($F_{ST} = 0.490$) was between Pebble Beach and Redwood Regional Park. The intervening area is likely to be inhospitable to both dispersal and settlement, due to habitat fragmentation and urbanization. For habitat specialists like *Zootermopsis*, population fragmentation can result in a reduction in effective population size, subjecting populations to the opposing forces of selection and genetic drift, which over a relatively short-time frame may lead to significant population divergence (Avise, 2004), as observed here. This is in sharp contrast to the low levels of population differentiation in more generalist species, such as those in the genus *Reticulitermes* (Vargo and Husseneder, 2011).

Our results contrast with those of Aldrich (2005), who found evidence of substantial gene flow between colonies of *Z. n. nevadensis* at different geographic locations up to distances of 170 km. An important difference between the present study and that of Aldrich (2005) was the degree of fragmentation of the study areas. Aldrich's study was performed in a location with significant connectivity between sites (in and around National Forests in far northern California), but our study involved sites fragmented both by urban development and natural barriers. Therefore, while distances between sites in the two studies are comparable, the dispersal pressure is likely to be significantly greater in the *Z. angusticollis* populations we studied. The higher level of genetic differentiation in the present study on *Z. angusticollis* compared to the earlier estimates for *Z. nevadensis* may also reflect differences in dispersal abilities of the two species.

Strong genetic differentiation at the spatial scales studied here appears to be unusual for termites, although to date there are data for only eight other species representing three families (Vargo and Husseneder, 2011). Strong genetic differentiation ($F_{ST} = 0.42$–0.62) has also been reported for the basal Australian termite, *M. darwiniensis* over distances of 2–400 km (Goodisman and Crozier, 2002). Likewise, Dupont et al. (2009) observed significant differentiation ($F_{ST} = 0.16$–0.25) among populations of the soil-feeding higher termite *Labiotermes labralis* (Family Termitidae) over distances of 30–120 km. These authors attributed the significant levels of population differentiation to habitat fragmentation and human disturbance.

Comparative colony genetic structure and breeding system

Similar to other studies of termite genetics, we found a mixture of colony types present. All populations had a mix of simple families and extended families, ranging from mostly simple families (64% in the Pebble Beach population of *Z. angusticollis*) to relatively few simple families (16% of the colonies in the Redwood Regional Park population of *Z. angusticollis*). Mixed family colonies were found only in *Z. angusticollis*: one colony in the Redwood Regional Park population, and two colonies in the Huddart County Park population. The population of *Z. n. nuttingi* consisted primarily of extended families (67%), with the remainder being simple families. Thus, extended families were the dominant colony type in all but the Pebble Beach population of *Z. angusticollis*. Extended families develop upon the death of one or both of the primary reproductives with replacement reproductives arising from their offspring (Myles, 1986; Thorne, 1997). Thorne et al. (2003) showed that developing colonies of *Z. nevadensis* nesting in the same log commonly interact, often resulting in the death of one or both sets of primary reproductives and eventual colony fusion. The result is a mixed family colony with new replacement reproductives from one or both colonies (Johns et al., 2009). The genetic signature of such mixed family colonies, i.e., the existence of five or more alleles at a locus, would continue to be present as long as workers from the two original colonies survived or as long as there continued to persist at least one reproductive from one original colony and two or more from the other original colony. The three mixed colonies of *Z. angusticollis* in the present study were

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likely formed through colony fusion. A similar, low percentage of mixed family colonies was detected by Aldrich and Kambhampati (2007) for *Z. nevadensis*. However, it should be noted that if colonies fuse at an early stage and the replacement reproductives originate from only one of the original colonies, or each colony supplies just one member of a reproductive pair, then the genetic diversity of all the new individuals produced in the colony will be limited and over time the signature of colony fusion will disappear through attrition. In such cases, genetic analysis of large, established colonies, as was performed in the present study, will underestimate the true extent to which colonies may undergo fusion at some point in their life cycle.

The strongly negative F_{IC} values obtained (all $F_{IC} \leq -0.121$) for the extended families in this study suggest that the mean number of replacement reproductives in our colonies was low, on the order of five or fewer, as indicated from simulations in Thorne et al. (1999). Aldrich and Kambhampati (2007) reported similar values in their study of a population of *Z. n. nuttingi* and a population of *Z. n. nevadensis*, suggesting that a relatively low number of effective reproductives within extended family colonies may be characteristic for the genus. However, the number in field colonies may be greater than this, with a maximum of 110 neotenics reported from one field colony of *Z. angusticollis* (reviewed in Myles, 1999). This is similar to many populations of subterranean termites, especially *Reticulitermes* spp., in which the effective number of reproductives in extended family colonies tends to be low, although the actual number can reach into the hundreds (reviewed in Vargo and Husseneder, 2009).

All four of our study populations contained simple families, and in all cases, the average degree of inbreeding in these colonies was relatively high (all $F_{IT} \geq 0.13$), indicating the reproductives heading colonies were on average close relatives. However, analysis of individual pairs gave a more complex picture, with colonies containing pairs of unrelated reproductives in some cases, while others contained highly related individuals. Differences in the age of the studied colonies could explain the observed variation in the degree of relatedness between pairs of reproductives. During tandem pair formation, primary reproductives of *Z. nevadensis* tend to pair with unrelated individuals (Shellman-Reeve, 2001). Assuming this is also true of *Z. angusticollis*, younger colonies headed by primary reproductives contained a king and queen that were unrelated. Older colonies may replace one or both of the primary reproductives with a single neotenic of the same sex, resulting in simple family colonies headed by close relatives. On the other hand, Rosengaus and Traniello (1993) found that laboratory established incipient colonies of *Z. angusticollis* comprised of nestmate primary reproductives were more likely to survive than those headed by unrelated reproductives, possibly through exposure to novel pathogens harbored by unrelated individuals. Similar results have been reported by Calleri et al. (2005) and Fei and Henderson (2003). If sibling headed colonies are also favored in the field, then such outbreeding depression could lead to a disproportionate number of established colonies headed by siblings than would be suggested by analysis of tandem pairs formed after mating flights. In *Z. nevadensis*, Aldrich and Kambhampati (2007) also found significant inbreeding within simple family colonies. Thus inbred colonies headed by monogamous pairs of related reproductives may be common in this group, arising through either outbreeding depression and/or replacement of the primary reproductives by a single pair of neotenics.

Our results here on *Z. angusticollis* and *Z. n. nuttingi* together with those of Aldrich and Kambhampati (2007) on *Z. n. nevadensis* and *Z. n. nuttingi* provide the first detailed analysis of the breeding structure of this basal genus of termites. Both studies show highly variable colony breeding structures within most populations including outbred simple families, inbred simple families, inbred extended families, and a small percentage of mixed family colonies. A similar mixture of family types also exists in the basal termite *M. darwiniensis* (Goodisman and Crozier, 2002), as well as many more derived termites, especially the subterranean termites (Vargo and Husseneder, 2009, 2011), suggesting that plasticity in colony breeding structure is ancestral in termites and continues to be advantageous for many species, even those with very different nesting and feeding habits. This plasticity should allow for populations to respond adaptably to local ecological conditions. In this regard, it is of interest to note that the Pebble Beach population was the only population of the three *Z. angusticollis* populations studied here to have a majority of simple families. The Pebble Beach site is an exposed coastal site dominated by Monterey pine, and thus is quite different from the more sheltered redwood forests of the other two sites, providing for the possibility that the differences in the observed breeding structures is due to differing ecological pressures. Given the limited gene flow between sites we documented, there could be ample opportunity for selection to act on populations and shape colony breeding structures depending on local conditions, although present levels of gene flow may be far less than they were historically, considering the relatively recent and drastic changes in habitat connectivity due to anthropogenic effects. Long-term studies across a wider range of locations are needed to determine whether differences in colony-breeding structure between sites is due to local environmental conditions, and, if so, to determine what specific ecological factors are most important in shaping colony breeding structure in *Zootermopsis*.

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