GENETIC ANALYSIS OF BREEDING STRUCTURE IN LABORATORY-REARED COLONIES OF *RETICULITERMES FLAVIPES* (ISOPTERA: RHINOTERMITIDAE)

CATHERINE E. LONG[1], EDWARD L. VARGO[2], BARBARA L. THORNE[3] AND THOMAS R. JUBA[2]
[1]American Pest Management, Inc., 6460 New Hampshire Avenue, Takoma Park, MD 20912, USA

[2]Department of Entomology, 3312 Gardner Hall, North Carolina State University, Raleigh, NC 27695, USA

[3]Department of Entomology, 4112 Plant Science Bldg., University of Maryland, College Park, MD 20742, USA

Primary reproductives, or kings and queens, within *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) colonies suppress sexual maturation of their offspring (Lüscher 1961). In the absence of this influence, immature individuals may differentiate into replacement reproductives (neotenics) (Pickens 1932; Esenther 1969; Howard & Haverty 1980; Thorne 1996). Snyder (1920) speculated that these neotenic individuals may leave the main nesting area with a small group of workers in order to establish distinct bud nests. To evaluate whether colonies containing neotenics would establish distinct daughter or bud nests within a network of physically separated but linked food resources, we provided laboratory colonies with 3, equal-volume food resources linked by 1-m sections of tubing. Termites were permitted to forage and move among the locations. After 20 months, workers were sampled from each of the 3 resources. Microsatellite analyses were performed to determine whether subpopulations within the resources exhibited distinct genotypic frequencies.

In 1993, incipient *R. flavipes* colonies were established in the laboratory with pairs of sibling alates collected from dispersal flights in Prince George's County, Maryland, USA (Thorne et al. 1997). In 2000, 13 of these colonies were transferred to their own three-resource feeding networks (Long et al. 2006 in press). All of these colonies retained their kings; 9 "queenright" colonies also contained a queen. In 4 "queenless" colonies, the founding queen had been replaced by at least 1 neotenic female 2-6 years prior to this experiment (Long et al. 2003).

Here we present data from Colony 1, a queenright colony (for simplicity, a single, representative sample is discussed), and the 4 queenless colonies (Colonies 2-5). DNA was extracted from 60 workers per colony, with 20 workers pulled from each food resource. Preparation and analysis of DNA followed Vargo (2003). Individuals were genotyped at seven microsatellite loci: *Rs 16, Rs 33, Rs 62, Rf 1-3, Rf 5-10, Rf 15-2,* and *Rf 24-2*. Twenty-one alleles were identified (Table 1); loci contained an average of 3 alleles. Average heterozygosity was 0.54 (0.31-0.90), a value comparable with those observed in North Carolina field populations (Vargo 2000; DeHeer & Vargo 2004).

Worker genotypes in the queenright colony and 3 of the 4 queenless colonies (Colonies 1-4) were consistent with those from simple families. However, locus *Rf 24-2* in Colony 5, which contained 14 neotenic females, contained 3 alleles in 5 genotypic classes; 4 homozygous genotypes were scored at *Rs 33*. Both scenarios are possible only if at least 3 and 4 parents, respectively, contribute to the offspring. Genotype frequencies alone cannot indicate exactly how many parents contribute.

Significant deviation from expected, homogeneous genotype frequencies for each locus were evaluated by a *G*-based test of differentiation among the subpopulations and then summed for an overall estimate of significance (Genepop 2004; Raymond & Rousset 2004). Only Colony 5 showed evidence of significant differentiation in genotype frequencies among the resources ($P < 0.0001$, $df = 12$).

The non-uniform distribution of Colony 5's alleles across the three-resource network suggests that differentiation may have a spatial component, either in offspring production or preferred distribution (i.e., associations of closest kin). At 2 loci, alleles or genotypes were not observed in all resources: at *Rs 33*, alleles *259* and *267* were missing in two resources, and the genotype *196/106* at locus *Rf 24-2* was absent from 1 of the sites.

In Colony 5, the resource in which workers harbored 2 unique alleles also contained the king, all 14 neotenic sisters, and all of colony's eggs and instars 1-3. Travel and mark-recapture data indicate that worker exchange occurred among all 3 sites throughout the colony's tenure in the three-resource network (Long 2005). Although the co-habitation of all reproductives does not suggest nest budding in this case, genetic isolation of a subset of workers that maintain constant contact with less genetically differentiated individuals lends support to the hypothesis that physical or functional budding can occur without complete isolation from nestmates (Thorne et al. 1999).

Our results provide a rare opportunity to evaluate the response of queenless colonies to a foraging arena consisting of physically separated but linked food resources. Even after 20 months, 3 of

TABLE 1. NUMBERS OF EACH GENOTYPE FOUND AMONG *R. FLAVIPES* WORKERS SAMPLED FROM 5 COLONIES. COLONY 1 WAS QUEENRIGHT; THE OTHERS WERE HEADED BY AT LEAST 1 NEOTENIC FEMALE. TWENTY-ONE ALLELES WERE IDENTIFIED AT 7 LOCI. MISSING DATA (—) INDICATE EITHER NON-SCORABLE PCR PRODUCT FOR THAT LOCUS OR THAT THE LOCUS WAS NOT SEQUENCED FOR THAT COLONY.

	Colony				
Locus genotypes	1[a]	2	3	4	5
Rs 16					
305/305	27		60	60	38
305/295	30	30			17
295/295		29			
Rs 33					
259/259	39	16	60	60	4
267/259		32			5
255/255					21
263/255					17
263/263					7
267/267	18	10			1
Rs 62					
315/315	57	60	21	19	55
319/319			9	9	
319/315			29	32	
Rf 1-3	—				
236/221			16		22
224/224			17		
224/221			16		
236/224			8		
224/218				4	
236/218					7
221/218		25			13
221/221					17
218/218		33			
245/245				2	
245/224				8	
245/218				7	
Rf 5-10	—				
153/153		59	60	31	24
153/147				25	33
Rf 15-2					
235/232		31	30	36	23
235/235	57		29	24	22
232/232		29			10
Rf 24-2					
106/106	12		30	1	10
196/106		31	30		6
169/106	12			15	20
169/169	12			27	6
196/169					16

[a]Sixty workers were examined from each colony. Failure of individual samples to yield readable data account for discrepancies between these totals and the number of genotypes presented.

TABLE 1. (CONTINUED) NUMBERS OF EACH GENOTYPE FOUND AMONG *R. FLAVIPES* WORKERS SAMPLED FROM 5 COLONIES. COLONY 1 WAS QUEENRIGHT; THE OTHERS WERE HEADED BY AT LEAST 1 NEOTENIC FEMALE. TWENTY-ONE ALLELES WERE IDENTIFIED AT 7 LOCI. MISSING DATA (—) INDICATE EITHER NON-SCORABLE PCR PRODUCT FOR THAT LOCUS OR THAT THE LOCUS WAS NOT SEQUENCED FOR THAT COLONY.

	Colony				
Locus genotypes	1[a]	2	3	4	5
199/106	21				
196/196		28			
199/169				16	

[a]Sixty workers were examined from each colony. Failure of individual samples to yield readable data account for discrepancies between these totals and the number of genotypes presented.

the 4 queenless colonies were genetically homogeneous. The genotypes sampled from the fourth queenless colony, which contained 14 female neotenics, indicate that genetic differentiation had begun to develop among the resources.

SUMMARY

Thirteen laboratory-reared *R. flavipes* colonies were housed in 3-resource foraging arenas for 20 months. Four of these colonies were queenless, having lost their founding queen 2-6 years prior. Microsatellite analysis performed on workers sampled from each resource allowed each colony to be classified as either a simple or an extended family and to examine the queenless colonies for evidence of genetic differentiation among the 3 linked feeding resources.

F-statistics (Wright 1921) and relatedness coefficient (*b*) (Pamilo 1984) were generated with Genetic Data Analysis software (Lewis & Zaykin 2001) with notational conventions of Thorne et al. (1999) and Bulmer et al. (2001). Among the 4 queenless colonies, F_{IT} = 0.52 (*c.i.* 0.37-0.65), F_{CT} = 0.59 (*c.i.* 0.48-0.69), F_{IC} = -0.17 (*c.i.* -0.27-0.08), and b = 0.78. These results are not significantly different from values predicted for an inbred colony with 2 female neotenics and a single male (Thorne et al. 1999). F_{IT} = 0.52 and b = 0.78 indicate marked inbreeding in this laboratory population. The founding of these colonies by probable siblings undoubtedly accounted for a portion of this observed loss of heterozygosity, but regional variation in levels of inbreeding may have also contributed (Reilly 1987; Bulmer et al. 2001; Vargo 2003). F_{IC} = -0.17 suggests an intermediate loss of heterozygosity within each colony, but not existence of differentiated bud nests. F_{CT} = 0.59 in this laboratory population indicates relatively high contrast between colonies.

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