Association between caste and genotype in the termite *Mastotermes darwiniensis* Froggatt (Isoptera: Mastotermitidae)

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Abstract　　Termite workers and soldiers differ markedly in their morphology and behaviour. We sought evidence for genetic influences on caste determination in the giant northern termite, *Mastotermes darwiniensis*, by investigating if workers and soldiers from the same colony differed genetically. The genotypes of 795 termites from 11 distinct colonies were assayed at six polymorphic microsatellite loci. We found that the multilocus genotypes of workers and soldiers from 8 of the 11 colonies did not differ significantly. Thus, the majority of the data provided no evidence for a genetic association with caste in workers and soldiers of *M. darwiniensis*. However, the genotype frequencies of workers and soldiers from three colonies differed, suggesting that genotype is occasionally associated with caste in this species. The genetic differentiation of castes within these colonies could reflect differences in the propensities of termites with distinct genotypes to develop into particular castes and provide a selective advantage to colonies headed by multiple reproductives.

Key words　　DNA microsatellite, eusociality, polygyny, population genetics, social insect.

INTRODUCTION

Social insect colonies are frequently headed by several reproductives or by reproductives that mate multiple times (Crozier & Pamilo 1996). Both of these situations typically lead to a decrease in nestmate relatedness, which may lower the inclusive fitness benefits of individuals undertaking helping behaviour within the colony. However, this apparent disadvantage may be countered by selection for increased genetic diversity within colonies if variation in the composition or performance of castes is associated with individual genotype (i.e., genetic polyethism) and such variation increases colony fitness. These conditions may arise when individuals with different genotypes are predisposed to developing into distinct castes or exhibiting specific behaviours.

Evidence for genetic polyethism has been discovered in several social Hymenoptera (Robinson & Page 1988; Stuart & Page 1991; O'Donnell 1998; Fraser *et al.* 2000). In addition, Kaib *et al.* (1996) found evidence for genetic polyethism in the Isoptera, where foraging *Schedorhinotermes amanianus* workers differed genetically from non-foragers. These results taken together suggest that genetic polyethism may be prevalent in social insects. However, data from more isopteran taxa are required to determine if the phenomenon is widespread. A strong candidate for further study is the termite *Mastotermes darwiniensis* Froggatt.

Mastotermes darwiniensis is the sole representative of the family Mastotermitidae and is considered to be one of the most primitive members of the Isoptera (Watson & Gay 1991), with molecular evidence indicating it to be the sister group to all other termites (Thompson *et al.* 2000). Colonies of *M. darwiniensis* are initiated by winged, adult imagoes, which function as the primary reproductives within the colony. In addition, colonies accept secondary (neotenic) reproductives as the colony grows (Watson & Abbey 1985; Watson & Gay 1991). However, the vast majority of termites within the colony belong to the worker and soldier castes. Workers and soldiers differ substantially in morphology and behaviour. Workers have relatively unspecialised mouthparts used for food acquisition and distribution. In contrast, soldiers have highly modified mandibles used to repel colony intruders (Watson & Gay 1991).

In this investigation, we tested for evidence of genetic polyethism in *M. darwiniensis* by determining if workers and soldiers sampled from the same colony differed genetically. Significant genetic differentiation between workers and soldiers would be consistent with an association between genotype and caste within colonies, and would provide evidence for genetic effects on caste. Moreover, such data would bolster the hypothesis that the presence of multiple reproductives within *M. darwiniensis* colonies may enhance colony function by increasing diversity among nestmates.

MATERIALS AND METHODS

We collected *M. darwiniensis* workers and soldiers from five locales [N-1, N-4, N-5, N-7, and N-11 described by Goodisman & Crozier (2002)] in the Northern Territory, Australia. All

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locales were separated by at least 15 km. A mean (± SD) of 19.61 ± 1.73 workers and 8.79 ± 8.25 soldiers were sampled from 5.60 ± 2.70 trees separated by about 1–100 m in each of these locales (Table 1). We assayed the genotypes of all 549 workers and 246 soldiers at the microsatellite loci Mdar2, Mdar3, Mdar4, Mdar5, Mdar8, and Mdar13 (Goodisman *et al.* 2001). The variability of each of the loci across all populations was quantified by the expected heterozygosity, $1 - \sum_i p_i^2$, where p_i is the frequency of allele i, as calculated by the program RELATEDNESS 4.2 (Queller & Goodnight 1989).

Previous genetic analyses of the workers collected in this study revealed that workers sampled from distinct trees frequently belonged to single colonies (Goodisman & Crozier 2002). Consequently, we attempted to group the workers and soldiers sampled in this study into colonies as well. To accomplish this goal, the genotypic frequencies of termites (workers and soldiers) from all pairs of trees were compared using an exact test as implemented by the program GENEPOP 3.2 (Raymond & Rousset 1995). Significant differentiation of termites collected from distinct trees indicated that the termites belonged to separate colonies, while non-significant differentiation suggested that they were part of the same colony.

Table 1 Numbers of *Mastotermes darwiniensis* workers (N_W) and soldiers (N_S) sampled from 28 trees in five locales from the Northern Territory

Locale	Tree	N_W	N_S
N-1	1a	11	1
	1b	20	5
	1c	20	4
	1d	20	4
	1e	20	12
	1f	20	7
N-4	4a	20	20
	4b	20	16
	4c	20	19
	4d	20	5
	4e	20	12
	4f	18	11
	4g	20	20
	4h	20	37
N-5	5a	20	16
	5b	20	6
	5c	20	1
	5d	20	9
	5e	20	1
	5f	20	12
N-7	7a	20	1
N-11	11a	20	6
	11b	20	1
	11c	20	2
	11d	20	3
	11e	20	4
	11f	20	9
	11g	20	2

We next determined if a single mating pair could have produced the genotypes of termites within colonies. Consideration of the number of reproductives within colonies may be important, because the presence of multiple reproductives may increase the probability of detecting a genetic component to caste if different reproductives are responsible for the production of distinct castes. The genetic data indicated that more than two reproductives were responsible for termite production within colonies if the assayed termite genotypes were inconsistent with the Mendelian segregation of alleles of a single reproductive pair at any of the six microsatellite loci.

The program RELATEDNESS 4.2 was used to calculate the relatedness (r) of termites belonging to distinct castes within colonies. This value provided a quantitative point estimate for the differentiation of soldiers and workers. Individual colonies were considered as 'demes' to control for allele frequency differences among colonies, and standard errors for the relatedness estimates were obtained by jack-knifing over loci. We used a t-test to determine if the estimate differed statistically from 0.0. An estimate significantly greater than zero would suggest that workers and soldiers differed genetically.

We next used the program GENEPOP 3.2 to conduct exact tests on the genotypic data in order to determine if workers were genetically differentiated from soldiers within individual colonies. Sequential Bonferroni corrections were used to adjust the rejection region for the multiple tests performed (Rice 1989). The individual P-values obtained from each colony-level comparison were then combined to obtain an overall estimate of the significance of differentiation for all colonies using Fisher's method of combined probability (Sokal & Rohlf 1995).

RESULTS

The expected heterozygosities across all sites for Mdar2, Mdar3, Mdar4, Mdar5, Mdar8, and Mdar13 equaled 0.63, 0.69, 0.32, 0.79, 0.72, and 0.48, respectively. Thus, these loci provided substantial power for detecting multiple reproductive lineages and genetic differentiation between castes.

Termites from the 28 distinct trees were successfully grouped into colonies in all four locales where more than one tree was sampled (Table 2). In all cases, the resulting colony designations corresponded exactly to those found by Goodisman & Crozier (2002), who had only used worker genotypes to group termites into colonies.

The genotypes of workers and soldiers within colonies were next examined directly to determine if colony mates were produced by more than a single pair of reproductives. Our results indicated that more than two reproductives were necessary to explain the observed worker and soldier genotypes in 72.7% of the colonies (Table 3). We note that this value represents an underestimate of the fraction of colonies containing more than two reproductives, because our ability to detect lineages was constrained by the variation of our

markers, the finite sample sizes, and the fact that reproductives within *M. darwiniensis* colonies are related (Watson & Abbey 1985). Nevertheless, the observed high proportion of colonies headed by multiple reproductives is consistent with the known biology of *M. darwiniensis* (Hill 1942; Watson & Abbey 1985), and, of more importance to this study, may increase the probability of detecting a genetic component to caste.

Finally, we turned our attention to the genetic differentiation of workers and soldiers within colonies. We first estimated the relatedness of termites belonging to distinct castes. Our estimate, 0.111 ± 0.031, differed significantly from zero ($t_{10} = 3.58$, $P = 0.005$), suggesting that workers and soldiers were genetically differentiated in at least some colonies.

Exact tests of genotypic differentiation between workers and soldiers helped distinguish which colonies were responsible for the high relatedness estimate. We found significant genetic differentiation between the castes in locale N-5 only (Table 4). However, we note that only the *P*-value from colony 5–2 was significant after correcting for the multiple tests performed; the small *P*-values obtained in colonies 5–1 and 5–5 can only be considered marginally significant. The differentiation of workers and soldiers in colony 5–2 was

evident at four of the five loci which were variable in termites sampled from this colony (*P*-values for Mdar2, Mdar3, Mdar5, Mdar8, and Mdar13 were <0.0001, 0.34, 0.00035, 0.0046, and 0.0017, respectively; Mdar2 was monomorphic) indicating that the significance of the test was not caused by the effects of a single locus. The significant and marginally significant *P*-values obtained in locale N-5 were sufficient to yield a significant overall estimate of genotypic differentiation between the castes ($P = 0.0004$), when the *P*-values from all colonies in all locales were combined.

DISCUSSION

In this investigation, we sought evidence for genetic influences on the composition of castes in the primitive termite *M. darwiniensis* by comparing the genotype frequencies of workers to soldiers within colonies. Significant genetic differentiation between the castes would be consistent with the hypothesis that termites of certain genotypes develop into distinct castes in this species. We found that workers and soldiers from most colonies did not differ genetically. However, in one locale, the genotype frequencies of workers and

Table 2 *Mastotermes darwiniensis* colonies detected in five locales from the Northern Territory. Numbers of workers (N_W) and soldiers (N_S) per colony are given, along with the tree designations from which the termites were obtained (see Table 1)

Locale	Colony	Trees	N_W	N_S
N-1	1–1	1a, 1b	31	6
	1–2	1c, 1d, 1e, 1f	80	27
N-4	4–1	4a, 4b, 4c, 4d, 4e, 4f, 4g, 4h	158	140
N-5	5–1	5a	20	16
	5–2	5b	20	6
	5–3	5c, 5d	40	10
	5–4	5e	20	1
	5–5	5f	20	12
N-7	7–1	7a	20	1
N-11	11–1	11a, 11b, 11c, 11f, 11g	100	20
	11–2	11d, 11e	40	7

Table 3 *Mastotermes darwiniensis* colonies in which multiple reproductive were (Y) or were not (N) required to account for the genotypes of workers, soldiers, or all termites combined

Locale	Colony	Workers	Soldiers	All termites
N-1	1–1	Y	Y	Y
	1–2	Y	Y	Y
N-4	4–1	Y	Y	Y
N-5	5–1	Y	Y	Y
	5–2	Y	N	Y
	5–3	N	N	N
	5–4	Y	N	Y
	5–5	Y	Y	Y
N-7	7–1	N	N	N
N-11	11–1	Y	Y	Y
	11–2	N	N	N

Table 4 Significance of genotypic differentiation of workers and soldiers from 11 *Mastotermes darwiniensis* colonies

Locale	Colony	*P*-value
N-1	1–1	0.698
	1–2	0.439
N-4	4–1	0.130
N-5	5–1	0.005
	5–2	< 0.0001
	5–3	0.342
	5–4	0.978
	5–5	0.015
N-7	7–1	0.570
N-11	11–1	0.440
	11–2	0.407

soldiers from a single colony differed significantly, while termites sampled from two other colonies within the locale showed marginally significant differentiation. These data suggest that genotype may occasionally affect determination of some castes in *M. darwiniensis*.

Colonies showing evidence for genetic associations with caste (5–1, 5–2, and 5–5) were headed by more than a single pair of reproductives (Table 3). This result is consistent with expectations. The presence of multiple reproductives should increase the number of genotypic combinations within colonies, thereby allowing for a greater opportunity for the expression of caste by genotype interactions. However, it should be noted that reproductives within *M. darwiniensis* colonies tend to be related due to the effects of inbreeding (Watson & Abbey 1985; Goodisman & Crozier 2002), thereby reducing the genetic variation within colonies relative to social insects whose colonies are headed by unrelated reproductives (Crozier & Pamilo 1996).

It is unclear why a significant effect was documented in colonies from locale N-5 only. One possibility is that we lacked power to detect genetic differentiation between the castes in other locales due to lower sample sizes or variability of markers within those locales. However, the number of termites sampled per colony was not necessarily lower in other locales (Table 2), nor were the microsatellites markers less variable (Goodisman & Crozier 2002). It is possible that colonies from the other locales differed from those in locale N-5 in unknown, yet important, ways. For example, genetic polyethism may be more likely to be detected when many reproductives are present, as may be the case with colonies located in disturbed habitats or with relatively old colonies (Watson & Abbey 1985). The former option would appear unlikely in explaining the variation among locales, as all samples were obtained from disturbed environments. The latter suggestion remains possible as colonies from the different locales may have differed in age. Unfortunately, the ages of most of the sampled colonies were unknown, and this possibility cannot be tested.

The genetic associations with caste documented in our study could arise for several reasons. First, it is possible that colonies exposed to distinct environmental conditions differ in the ratio of workers to soldiers they produce, and that multiple colonies were accidentally sampled from single trees in this study and considered as belonging to the same colony. This possibility seems unlikely, as our ability to group *M. darwiniensis* termites sampled from distinct trees into colonies based on genetic data precludes extensive mixing of termites from different colonies within trees.

Second, the observation of genetic differentiation between workers and soldiers may arise if there has been a recent transition in the reproductives present in the colony, and if soldiers and workers show differential mortality or developmental rates (Fraser *et al.* 2000). The likelihood of this explanation is difficult to assess in *M. darwiniensis*. The relative mortality rates of the castes are unknown and the system is further confounded by the fact that soldiers are developmentally derived from workers (Watson *et al.* 1977).

A third explanation for the observed genetic patterns is that termites of certain genotypes may be biased towards developing into particular castes. Evidence for a genetic basis to behaviour of castes already exists in other social insects (Robinson & Page 1988; Stuart & Page 1991; Kaib *et al.* 1996; O'Donnell 1998; Fraser *et al.* 2000). In general, such genetic polyethism may be selectively advantageous because it would allow members of a colony to readily undertake a wide range of behaviours, thereby increasing overall colony fitness. Enhanced colony fitness through increased genetic diversity could select for social insect colonies whose members were produced by multiple reproductives (Crozier & Pamilo 1996) and counteract potential detrimental effects, such as a reduction in colonymate relatedness, associated with the presence of multiple genetic lineages.

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