

# Fine-Scale Genetic Structure and Fire-Created Habitat Patchiness in the Australian Allodapine Bee, *Exoneura nigrescens* (Hymenoptera: Apidae)

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## Abstract

Fire promotes an abundance of nest sites for the stem nesting bee *Exoneura nigrescens*, which remain viable for approximately 10 years. The finite duration of nesting substrate and localized fire events suggest that migration should minimize genetic structure among suitable habitat patches. *Exoneura nigrescens* was sampled from 7 localities with a known fire history in southwestern Victoria, Australia. Individual bees were genotyped at 8 microsatellite loci and genic and genotypic analyses applied to examine genetic structure among burn patch localities, within burn patches, and within colonies. Despite relatively short-term availability of nesting substrates, remarkably fine-scale genetic structure was observed both among burn patches and within burn patches. The spatial distribution of relatedness shows a strong pattern of isolation-by-distance at geographic distances to 35 km, suggesting that genetic partitioning among burn patches is, at least in part, a result of dispersal ability. Genetic structure within burn patches includes colonies consisting of close kin with genic partitioning among nests. Relatedness structure within colonies suggests that polygamy, multiple breeding pairs, and a lack of inbreeding typifies the mating system.

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The degree of gene flow between areas of suitable habitat influences the level of genetic differentiation among, and genetic variation within, habitat patches (Frankham et al. 2002). Small isolated populations are especially susceptible to loss of genetic variation through drift which, in turn, can increase the risk of inbreeding depression and extinction (Saccheri et al. 1998; Taylor 2003). In social insects, these detrimental effects are expected to be more pronounced because many individuals forgo reproduction thus reducing the effective population size. In addition, inbreeding within several hymenopterans has been shown to result in sterile diploid males (Stahlhut and Cowan 2004), which further reduces the effective population size.

Although inbreeding may have detrimental effects, inclusive fitness benefits may actually favor inbreeding and be instrumental in the evolution of highly social inbred organisms (Hamilton 1964a, 1964b; Bourke and Franks 1995). Clearly, knowledge of relatedness structure is necessary to start unraveling selective pressures involved with the evolution of sociality (e.g., Bourke 1997; Pamilo et al. 1997; Tóth et al. 2002). Relatedness structure has been assessed in many hymenopterans, including wasps (e.g., Stahlhut and Cowan 2004), ants (e.g., Sundström et al. 2003), and bees (e.g., Paxton 2000; Cameron et al. 2004). Allodapine bees are an ideal taxon

in which to identify the factors important to the evolution of sociality as they display large variation in group structure both inter- and intraspecifically (Hurst et al. 1997; Cronin and Schwarz 1999a, 1999b; Tierney et al. 2002; Langer et al. 2004a). Further, contemporary environmental and genetic influences on social structure can be disentangled from phylogenetic conservatism because the clade to which all species belong is monophyletic and the common ancestor was social (Schwarz et al. 2003).

The subject of this study, *Exoneura nigrescens* (previously referred to as *Exoneura bicolor*), is an allodapine bee that displays substantial variation in group structure (e.g., Schwarz et al. 1998). Colonies of *E. nigrescens* can vary from solitary, with a single female and her brood, to primitively eusocial, consisting of 2 generations of adults caring for brood (see Schwarz 1987 and Silberbauer and Schwarz 1995). Although the extent to which task specialization remains unclear, several advantages of being part of a large group of related individuals have been identified for *E. nigrescens*, including reduced reproductive skew and higher levels of survival (Schwarz et al. 1998; Langer et al. 2004a). Nevertheless, major variation in group structure exists (e.g., Hogendoorn and Zammit 2001), suggesting that other factors are involved

in molding within-colony structure. For these reasons, *E. nigrescens* has been adopted as a model species to investigate the processes involved in the evolution of sociality (e.g., Schwarz 1987; Schwarz et al. 1998; Langer et al. 2004a).

*Exoneura nigrescens* excavates nests in any suitable pithy stem throughout its distribution in southeastern Australia. In our study areas in southwestern Victoria, *E. nigrescens* predominately nests in the inflorescence stalks of *Xanthorrhoea minor* and excavates a round hole at the top of the inflorescence, below which is a tunnel that lengthens over time. The flowering spikes of *Xanthorrhoea* generally develop after fire (Bedford 1985) and then gradually disintegrate, rarely remaining intact for more than 8 or 9 years (Silberbauer and Schwarz 1995). The extent to which any variation in colony structure is related to the duration of nest occupancy remains untested and is one of the aims of the present study.

Because woodland fires are patchy, *Xanthorrhoea* nesting sites are also patchy in both space and time. Thus, the distribution of *E. nigrescens* approximates a metapopulation (Gilpin 1987), although it should be noted that this structure may be eroded to an extent by *E. nigrescens* occasionally nesting in other vegetation types (e.g., *Melaleuca*, Silberbauer L, personal observation). Nevertheless, examining how genetic variation is distributed may reflect the relative importance of habitat patchiness, population size, and isolation to inbreeding (as opposed to potential behavioral mechanisms associated with inclusive fitness). It may also be valuable in determining the potential consequences of anthropogenic habitat fragmentation on genetic variability in social organisms. By virtue of their social structure, these bees have especially small effective population size/census size ( $N_e/N$ ) ratios (e.g., Graur 1985). *Exoneura nigrescens* may be at particular risk of losing genetic variability because, other than haplodiploidy and skewed sex ratios, habitat patches are subject to frequent extinction and recolonization, which will tend to reduce effective population even further (Frankham et al. 2002).

The regularity of fire in most terrestrial Australian environments places *E. nigrescens* among a large diversity of organisms with a metapopulation structure maintained by a particular fire frequency. Although burning is often used as a management tool for biodiversity conservation (Gill and McCarthy 1998), there is relatively little information on the effect of frequency on forest invertebrates (Gill and McCarthy 1998). In light of potential problems that may be associated with isolation in social insects, characterizing the spatial scale over which fire-dependent social invertebrates disperse provides information pertinent to fire management activities.

In short, the objectives of this study were to use microsatellite loci to examine population structure and within-nest relatedness of *E. nigrescens*. We assess the relationship between fire interval and colony characteristics including colony size and the extent of inbreeding within burn patches. The degree of connectedness among habitat patches will inform on the level to which inbreeding is mediated by behavior (e.g., by kin bias in mating partners) or limited gene flow. It will also provide information on whether anthropogenic habitat modification is likely to disturb natural patterns of gene flow, which in turn, could impact on genetic variation and population survival.

## Methods

### Sample Collection, DNA Extraction, and Microsatellite Typing

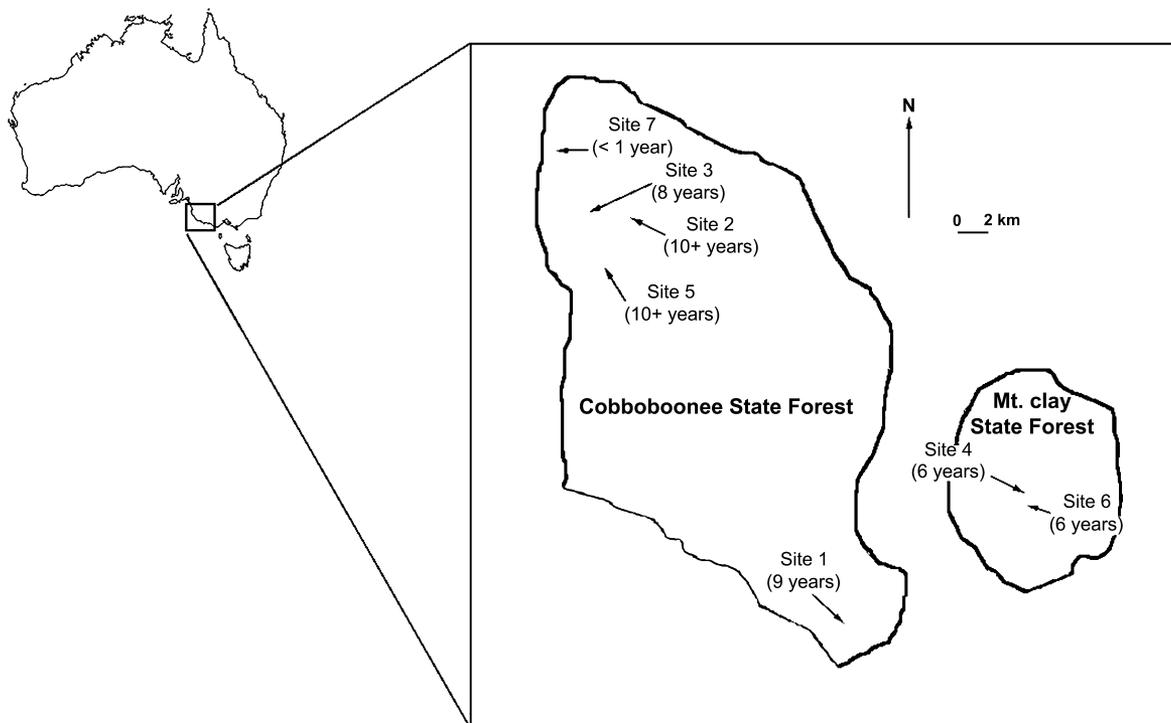
Intact colonies were collected in *Xanthorrhoea minor* inflorescences during February 2004 from Cobboboonee State Forest (38°0'S, 141°35'E) and Mt. Clay State Forest (30°10'S, 141°40'E) in western Victoria, Australia. Collection was carried out at temperatures below 15 °C as individuals do not leave their nests to forage at this temperature. Nest locations were determined using GPS (GARMIN 12XL). Nest entrances were blocked with cotton wool, bar coded, chilled to 4 °C and returned to Macquarie University, NSW. Colonies were collected from locations with known fire history (Figure 1), which are hereafter referred to as burn patches. Within each burn patch, the distances between colonies collected ranged from approximately 0.1 to 500 m. Within each burn patch, searching was thorough, and all located colonies were collected.

DNA from 402 bees within 89 nests was extracted via salting out (Sunnucks and Hales 1996) and genotyped at 8 microsatellite loci. PCR conditions for each of the 8 loci have been reported previously (Langer et al. 2004b), and none of these loci were known to possess null alleles or to deviate significantly from linkage disequilibrium in *E. nigrescens* (see below and also Langer et al. 2004b). The 3 levels at which genetic structure was characterized were 1) within colonies, 2) among colonies within burn patches, and 3) among burn patches. All individuals from 33 colonies (a total of 301 bees) were genotyped to obtain within-colony data. Eighteen of these colonies were collected from burn patch to have dual purpose with other research objectives. Whole colonies were randomly selected from several other localities to ensure that these data were representative. To avoid potential bias from sampling error and high within-colony relatedness, genotype data from only a single female per nest was used for all other analyses. Data from a single female were selected at random from each of the 33 colonies where all individuals were genotyped, whereas only a single female was genotyped from each of the remaining 56 colonies. Characteristics of the loci used are given in Table 1. Heterozygosity estimates were calculated using GENEPOP version 3.1d (Raymond and Rousset 1995) and  $F_{is}$ , with standard errors estimated via jackknifing, calculated using FSTAT version 2.9.3 (Goudet 2001).

A total of 41 male bees were identified by morphological characters and, after genotyping, were excluded from the following analyses. Because male bees are haploid, the presence of null alleles can be inferred from a lack of PCR product. PCR products generated for the 41 males gave no indication of null alleles, other than 4 individuals for which PCR consistently resulted in poor amplification across loci (presumably owing to poor DNA quality).

### Dispersal among Burn Patches

A powerful approach to assess fine-scale genetic structure and inferred dispersal is to exploit information from composite



**Figure 1.** Map showing each of the 7 sampling locations within Cobboboonee and Mt. Clay state forests and the time period since fire (given in parentheses).

genotypes across several loci (“genotypic analyses” see Sunnucks 2000), particularly if data are analyzed at the level of the individual (Sunnucks 2000; Stow et al. 2001). Recent statistical developments allow for genotypic autocorrelation to be evaluated at several different spatial scales. With these spatial autocorrelation analyses, more subtle patterns in genetic structure can be provided than with the traditional linear relationship tested by a standard Mantel test (Mantel 1967; Stow et al. 2001).

Dispersal patterns among burn patches were inferred by examining the geographical structure of relatedness by applying spatial autocorrelation analysis. Relatedness estimates ( $R$ ), derived from a multilocus measure of genetic distance be-

tween individuals (Smouse and Peakall 1999), were calculated at several distance classes using the software GenAIEx (Peakall and Smouse 2001). The  $R$  values generated should approximate those expected according to pedigree. Values obtained have been found to be equivalent to those calculated using softwares such as KINSHIP (Banks et al. 2005). The  $R$  values obtained range from +1 to -1 with a mean of zero (no autocorrelation). Positive  $R$  indicates greater genotypic similarity than expected by chance for the given data set, and negative values indicate less similarity. For each distance class, the significance of any deviation from zero was assessed by 999 permutations (Peakall and Smouse 2001). The 95% confidence intervals (CIs) around  $R$  were obtained via bootstrapping 999 times. The first distance class of the correlogram analyses pairwise  $R$  between all individuals sampled together at a particular burn patch. Levels of dispersal among patches were also evaluated by computing a genetic distance measure, analogous to  $F_{st}/(1-F_{st})$  (Rousset 2000), between each individual from one burn patch and every individual from all the other patches, and assessed for isolation-by-distance using Mantel testing with 5000 permutations, executed by GENEPOP version 3.1d (Raymond and Rousset 1995).

In addition to the individual-based analyses given above, the level of gene flow among sampling localities was also inferred with  $F$  statistics ( $F_{it}$ ,  $F_{is}$ ,  $F_{st}$ ) calculated using FSTAT version 2.9.3 (Goudet 2001). The relationship of  $F_{st}$  and geographic distance was assessed by Mantel testing, implemented by GENEPOP version 3.1d (Raymond and Rousset 1995).

**Table 1.** The number of alleles ( $K$ ), heterozygosity estimates, and  $F_{is}$  (SE) per locus, in addition to probability values of deviation from Hardy–Weinberg equilibrium. Data include only a single female per colony (from 89 colonies) and are pooled from the 7 sites

Locus	$K$	$H_e$	$H_o$	$F_{is}$	$P$ value
N39	22	0.873	0.724	0.081 (0.077)	<0.0001
R32	25	0.906	0.368	0.504 (0.070)	<0.0001
N81	34	0.880	0.544	0.333 (0.080)	<0.0001
R115	16	0.765	0.326	0.523 (0.125)	<0.0001
N60	37	0.957	0.551	0.425 (0.111)	<0.0001
N149	17	0.873	0.616	0.256 (0.052)	<0.0001
N83	27	0.950	0.581	0.401 (0.065)	<0.0001
R74	22	0.928	0.338	0.395 (0.079)	<0.0001

**Table 2.** The number of adults (*A*) and immature (*I*) *Exoneura nigrescens* within each of the 33 colonies from which all individuals were genotyped. Mean relatedness (*R*) calculated for each group is included

Group	<i>A</i>	<i>I</i>	Total	Whole group <i>R</i> (±SE)	Immature <i>R</i> (±SE)
1	14	8	22	0.67 (0.03)	0.63 (0.05)
1	14	6	20	0.57 (0.03)	0.60 (0.07)
1	15	4	19	0.59 (0.04)	0.64 (0.04)
1	5	2	7	0.27 (0.07)	0.46 (0.09)
1	10	4	14	0.61 (0.05)	0.56 (0.08)
1	2	5	7	0.51 (0.07)	0.57 (0.04)
1	10	10	20	0.51 (0.04)	0.58 (0.06)
1	5	5	10	0.43 (0.08)	0.38 (0.13)
1	3	0	3	0.49 (0.07)	NA
1	7	3	10	0.41 (0.05)	0.40 (0.03)
1	10	8	18	0.42 (0.05)	0.39 (0.06)
1	6	2	8	0.49 (0.08)	0.84 (NA)
1	14	5	19	0.30 (0.06)	0.47 (0.03)
1	5	1	6	0.37 (0.06)	NA
1	19	5	24	0.36 (0.04)	0.51 (0.04)
1	1	7	8	0.55 (0.06)	0.53 (0.06)
1	7	3	10	0.57 (0.07)	0.73 (0.09)
1	3	0	3	0.56 (0.15)	NA
2	0	2	2	0.58 NA	—
2	1	1	2	0.59 NA	NA
2	3	0	3	0.37 (0.28)	NA
4	1	1	2	0.39 NA	NA
4	1	1	2	0.42 NA	NA
4	1	2	3	0.59 (0.11)	0.59 (NA)
4	1	1	2	0.53 NA	NA
4	1	2	3	0.75 (0.04)	0.82
6	7	3	10	0.44 (0.08)	0.24 (0.16)
6	2	0	2	0.72 NA	NA
6	1	1	2	-0.09 NA	NA
6	2	3	5	0.15 (0.08)	0.22 (0.12)
6	1	1	2	0.15 NA	NA
7	1	2	3	0.65 (0.06)	NA
7	13	10	23	0.65 (0.03)	0.55 (0.03)

NA, not applicable.

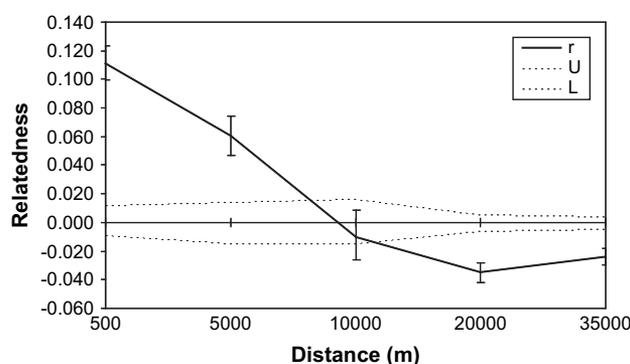
**Within-Colony Analyses**

Within-colony relatedness was estimated from the 33 nests from which all individuals were genotyped, and 2 or more individuals were present, using the Macintosh program KINSHIP version 1.2 (Goodnight et al. 1998). To prevent potential bias from close kin, individual pairwise *R* values were based on allele frequencies excluding data from bees sampled in the same nest as the 2 current target individuals (Goodnight et al. 1998). Summary data for within-nest colony size and age structure and relatedness values are given in Table 2.

**Results**

**Among-Burn Patch Genetic Structure**

Genetic structure was evident at a spatial scale that encompassed the 7 burn patches. Genotypic structure was clearly shown by the spatial distribution of relatedness illustrated by the correlogram (Figure 2). Significantly elevated genotypic similarity was apparent in the first 2 distance bins

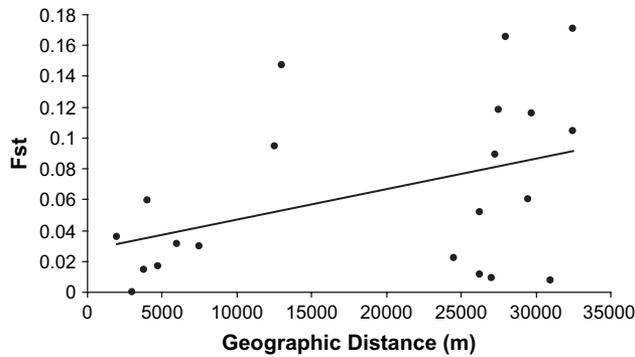


**Figure 2.** Correlogram of genotypic similarity (*R*) with geographic distance. The solid line tracks relatedness, dashed lines represent the upper (U) and lower (L) 95% CI around random expectations, whereas bars around *R* show the 95% CI determined by bootstrapping.

(0–0.5 km) and significant genotypic dissimilarity among individuals sampled in the last 2 distance classes (20–35 km). The presence of isolation-by-distance was supported by a moderate, but highly significant ( $P < 0.0001$ ), positive relationship between pairwise genetic distance among individuals and geographic distance. In addition to comparisons between pairs of individuals, partitioning of allele frequencies was evidenced by moderate  $F_{st}$  values obtained between several of the burn patches (Table 3). These also increase significantly ( $P = 0.034$ ) with geographic distance (Figure 3). However, we do not necessarily conclude isolation-by-distance from these  $F_{st}$  data. The positive relationship between  $F_{st}$  and distance is largely driven by genetic distances between 2 clusters of sampling localities separated by 20 or more kilometers (Figure 1). Correlations of  $F_{st}$  with geographic distance within each cluster are not significant ( $P > 0.05$ ). Indeed, even with our more highly resolving genotypic data, significant genetic dissimilarity was only evident between individuals sampled in different “clusters,” that is, at distances 20 km or farther. Nevertheless, these genotypic data do demonstrate isolation-by-distance with the 95% CIs around the correlation coefficients showing a significant decline in *R* for each distance class out to 20 km (Figure 2). The spatial distribution of *R* and genetic distance, along with  $F_{st}$  values, point to a spatial Wahlund effect (pooling data across a genetically subdivided population) explaining why each of the 8 microsatellite loci show a significant heterozygote deficit

**Table 3.** Matrix of pairwise  $F_{st}$  values between each sampling locality (probability values  $P < 0.05$  and  $P < 0.001$  are indicated by \* and \*\*, respectively)

Population	1	2	3	4	5	6
2		0.0385				
3		0.0089*	0.0502			
4		0.0944*	0.1305	0.0606		
5		0.0224*	0.0984	0.0144*	0.0517	
6		0.1475**	0.1925	0.1156*	0.0361*	0.1185**
7		0.0079	0.0680	0.0167	0.1042	0.0294* 0.1708**



**Figure 3.** Illustrates the positive relationship ( $P = 0.034$ ) between  $F_{st}$  and geographic distance.

(Table 1). Finally, nonrandom mating among burn patches is further supported by significantly less genetic variation within individuals relative to the total population ( $F_{it} = 0.443$ ; 95% CI 0.339–0.543).

### Within-Burn Patch Genetic Structure

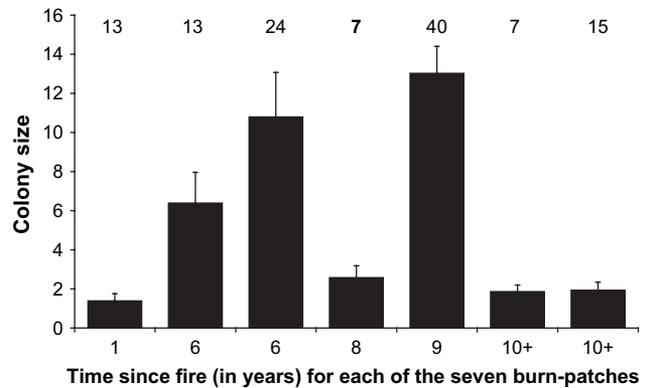
Analysis of genetic structure within burn patches reveals that only 1 of the 7 sampling localities (burn patch 2) did not deviate significantly from Hardy–Weinberg equilibrium after correction for multiple tests at  $\alpha = 0.05$  (Rice 1989; Table 4). Significant homozygote excesses were shown by positive  $F_{is}$  values and may be attributable to inbreeding and/or a Wahlund effect owing to social structure (Sugg et al. 1996; see Discussion).

### Within-Colony Genetic Structure

Considerable variation in within-colony relatedness was obtained [mean  $\pm$  standard deviation (SD);  $0.478 \pm 0.178$ , Table 2]. The average level of relatedness obtained among immature females of  $0.535 (\pm SD; 0.163)$  was substantially less than the theoretical value of 0.75 expected between females sharing the same parents in a haplodiploid system such as ours (Table 2). To ensure that our allele frequency data were providing the relatedness estimates expected according to pedigree, 1000 simulated female genotypes were generated from the very same allele frequency data used for

**Table 4.** Number of colonies ( $N$ ) from which genotype data was obtained in each of 7 “burn” patches, the time ( $T$ ) since the last fire, expected heterozygosity, and probability value for deviation from Hardy–Weinberg equilibrium

Population	$N$	$T$	$H_e$	$F_{is}$	$P$ value
1	19	9	0.805	0.401	<0.0001
2	6	>10	0.806	0.178	0.0391
3	10	8	0.688	0.384	<0.0001
4	11	6	0.795	0.575	<0.0001
5	12	10	0.825	0.346	<0.0001
6	20	6	0.564	0.530	<0.0001
7	11	1	0.698	0.186	<0.0001



**Figure 4.** Histogram (with standard error bars) illustrating the relationship between colony size (number of individuals) and the number of years since fire for each of the 7 burn patches. Sample sizes are given above each histogram bar.

these relatedness data (executed by KINSHIP version 1.2). An average relatedness value of  $0.747 (\pm SD; 0.048)$  was obtained between females derived from the same parents, thereby confirming that our relatedness estimates reflect pedigree. Differences between mean relatedness among immature female bees ( $0.535 \pm 0.163$  SD) and among adult female bees ( $0.490 \pm 0.148$  SD;  $P = 0.251$ ) was found to be nonsignificant (difference of means tested by randomization, using the software RT version 2.1, Manly 1997), suggesting that adult female bees exhibit a high degree of philopatry to their natal nest. Our estimate of relatedness among immature females is lower than that previously reported by Schwarz (1994) who found a relatedness value of 0.714 among female brood using allozyme data. However, average colony relatedness among adult females was found to be similar to values previously reported (relatedness  $\sim 0.475$ , Schwarz 1994; Schwarz et al. 1998). This discrepancy in relatedness among immatures is interesting because our average relatedness value of 0.535 suggests that *E. nigrescens* colonies were not predominately generated by a single breeding pair. Furthermore, multiple breeding pairs and/or polygamy were suggested if predispersal individuals from the same colony total more than 3 alleles at a particular locus. Of the 33 nests assessed, females from only 8 nests could have shared the same parents (having no more than 3 alleles at any locus). These data support previous research showing that related females are the preferred nest mates and that each contributes to brood (e.g., Langer et al. 2004a).

### Relationship between Fire Interval and Group Characteristics

Average colony size tends to increase with time since fire up to approximately 9 years, after which colony size tends to decrease (Figure 4). In addition, there is a strong positive relationship between nest length and colony size (analysis of variance;  $F = 89.158$ ,  $P < 0.0001$ ). Correspondingly, nest length also tends to increase with time since fire and then decrease in a similar fashion to colony size (data not shown).

The decline in colony size after 9 years is in agreement with the maximum time period that nests persist and *Xanthorrhoea* flower spikes take to decompose (Silberbauer and Schwarz 1995). The trend of increasing colony sizes with time since fire is weak and disrupted by relatively small colony sizes in a patch sampled 8 years after burning (Figure 4). Perhaps in this case, time since fire has been confounded with other factors, such as resource availability. For example, *Xanthorrhoea* occasionally flowers in response to factors other than fire, and perhaps, nesting availability in this patch reflects a *Xanthorrhoea* flowering event disassociated with burning.

Given the limited gene flow among patches (Figure 2), an increase in inbreeding with time since fire may have been expected. However, a relationship between the inbreeding coefficient,  $F_{is}$ , and time since fire was not evident (Pearson correlation  $P = 0.904$ ). There was, nevertheless, a nonsignificant trend toward higher levels of expected heterozygosity with time since fire (Pearson correlation  $P = 0.128$ , Table 4), possibly because occasional migration enriches allelic diversity over time.

## Discussion

The mosaic of burn histories and the fairly rapid turnover of available nesting sites with time suggests that regular colonization events may reduce spatial genetic structure in *E. nigrescens*. To the contrary, we found remarkably fine-scale genetic structure, both within and among burn patches. Limited dispersal between burn patches was inferred by very strong genotypic structure (Figure 2) as well as moderate structure in allele frequencies (Table 3). Genotypic structure also shows a pattern of isolation with distance with the spatial correlogram illustrating a marked and significant decline of relatedness with distance. This suggests that the genetic structure is, at least in part, the result of limits to dispersal ability as well as temporal variability in nesting site availability.

Within burn patches, significant heterozygote deficits (positive  $F_{is}$  values) could be potentially the result of genetic partitioning (the Wahlund effect) and/or inbreeding (Table 4). High levels of inbreeding have been demonstrated in other Hymenoptera (e.g., Sundström et al. 2003; Stahlhut and Cowan 2004). The mating system of *E. nigrescens* inferred from within-colony structure points to multiple mating and/or multiple breeding pairs, suggesting that if inbreeding is present, it is more likely a consequence of processes operating at broader scales than the colony. If inbreeding was commonplace within burn patches, as the time period since fire lengthened, expected heterozygosity is expected to decrease through erosion of allelic diversity (e.g., Frankham et al. 2002). Instead, we found a trend toward increased levels of expected heterozygosity. If Wahlund effect explains the lower than expected heterozygosity, it is unlikely that any within-patch genetic substructure is a result of dispersal limitations. Floral resources for *E. nigrescens* are patchily and sporadically distributed, and individuals forage over distances considerably greater than internest distances. A likely explanation is that social behaviors have generated the observed genetic pattern (e.g., Sugg et al. 1996). Previous work has

shown that *E. nigrescens* can assess relatedness, preferentially associates with other related females and, despite substantially compromised survival, would rather nest alone than with unrelated females (Langer et al. 2004a). Thus, genic structure at the burn patch level may arise from kin recognition and exclusion of unrelated females from nests, perhaps in conjunction with populations being established by queens from different source populations.

Corresponding to kin bias in colony-mate choice, mean within-colony relatedness was quite high, although a large range in relatedness values was obtained ( $-0.09$  to  $0.75$ ). A large variation of within-nest relatedness may be expected given the “tug of war” model described by Langer et al. (2004a) whereby female *E. nigrescens* can adapt their reproductive strategy according to 3 parameters as follows: 1) ecological constraints, 2) group productivity, and 3) genetic relatedness. High within-colony relatedness among females is favored because it minimizes reproductive skew, thereby maximizing productivity per individual. In addition, large colony sizes in *E. nigrescens* are associated with higher survival (Bull and Schwarz 1996; Schwarz et al. 1998; Hogendoorn and Zammit 2001).

Because of the critical role of fire in creating suitable habitat for *E. nigrescens*, it seems reasonable to suppose that social organization of this species evolved in a metapopulation structure. The ability to recognize and preferentially nest with close kin (Langer et al. 2004) suggests that, historically, the spatial scale over which burn patches have been distributed typically allowed for reasonable levels of interpatch dispersal and therefore appreciable genetic variation among colonies. Our finding of significant isolation-by-distance suggests that isolated burn patches may receive smaller numbers of founding individuals and less allelic enrichment through gene flow over time. In future studies, it would be of interest to investigate whether isolation is correlated with increasing patch-wide relatedness and whether variation in this trait leads to differences in social interaction and organization. For example, an increase in relatedness between colonies may result in an increase in natal dispersal of adult females (which our current data shows is low) as other factors, such as those suggested in the tug of war model by Langer et al. (2004), take precedence. Investigating the effects of isolation could also inform conservation management of Australian native bees subjected to anthropogenic habitat fragmentation, an area for which research is currently lacking (Repaci et al. 2006).

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