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Size and heterozygosity influence partner selection in the Formosan subterranean termite

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In monogamous species that exhibit extensive biparental investment, such as termites, both sexes are predicted to be selective when choosing a mate. Size-related traits are expected to be important in partner selection because the fat reserves of the colony founders sustain the incipient colony. Partner relatedness and heterozygosity determine the degree of inbreeding and genetic diversity within the colony and may thus also influence partner selection. To test these predictions, we investigated whether phenotypic and genetic traits influence mate choice and/or competitive advantage during pair formation of Formosan subterranean termites, *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Pair formation in termites normally occurs within a short period after swarming when alates form tandem pairs on the ground. Alates were collected from 5 light trap samples in the French Quarter of New Orleans, LA. From each sample, both tandem pairs and single individuals were collected and their sex, body weights, and head widths were recorded. Pairwise relatedness and individual levels of heterozygosity were determined by microsatellite genotyping. Males in tandem pairs with females had a significantly larger head width than males that did not form tandem pairs. Weights as well as head widths of tandem running partners were positively correlated. For the majority of the samples, relatedness between tandem partners did not differ from the relatedness to members of the other tandem pairs. Thus, no kin discrimination occurred during tandem running. However, females engaged in tandem running had a higher degree of heterozygosity than females that remained single. These findings suggest partner selection and/or competitive advantage based on size-related phenotypic parameters and genetic diversity. The pairing advantage of heterozygous females might explain previous findings of sex-biased alate production depending on the degree of inbreeding in colonies of several species of the genus *Coptotermes.* *Key words:* Isoptera, mate choice, microsatellite genotyping, morphometry, relatedness, Rhinotermitidae, sexual selection. *[Behav Ecol 19:764–773 (2008)]*

Partner selection

In monogamous species that exhibit extensive biparental investment, both sexes are predicted to be selective when choosing a mate (Trivers 1972). Whereas monogamy and equal parental investment are common in birds (Wittenberg and Tilson 1980), this system is rarely found in insects (Bonduriansky 2001). The exception to this rule are the termites (order Isoptera), which represent the second largest animal taxon with monogamous biparental care behind the birds. The majority of termite reproductives are monogamous during initial colony foundation; they mate for life and show extensive biparental investment in raising their offspring (Rosengaus and Traniello 1991; Shellman-Reeve 1999, 2001). Despite the fact that there are more than 2700 ecologically and economically important termite species, partner selection in termites has received little attention. In particular, the factors influencing partner selection and competitive advantages are largely unknown. Investigators of mating systems have noted this conspicuous absence of studies involving mating behavior and partner selection in termites (e.g., Alexander et al. 1997).

Phenotypic traits and partner selection

For most invertebrate species, phenotypic traits play key roles in mate selection (Bonduriansky 2001). Typical phenotypic traits considered important in mate choice include body weight and size, both assumed to be reasonable predictors of fecundity and investment capability. Although phenotypic traits are most often regarded as proximate cues for fecundity, they can also serve as possible indicators of genetic quality, such as the degree of inbreeding. For example, inbreeding depression can lead to stunted growth, which is reflected in decreased size and weight in social spiders and crickets (Bilde et al. 2005; Rantala and Roff 2005).

Parental investment is immense in termites because the first generation of larvae is entirely dependent on the resources of the founder pair (Shellman-Reeve 1999). For several weeks, the incipient colony is sustained solely by the fat reserves of the parents until foragers emerge to provide the young colony with nutrition. Therefore, it is conceivable that size and/or weight may play a role in mate selection in termites. Studies along these lines have been conducted on only 3 termite species. Shellman-Reeve (1999) found that in the dampwood termite, *Zootermopsis nevadensis* (Termopsidae), males choose heavy females (with large fat reserves) and females choose large males. Matsuura and Nishida (2001) reported that males of *Reticulitermes speratus* paired with heavier females. Kitade et al. (2004) showed that paired dealates in *Reticulitermes kanmonensis* tended to be larger in head width, pronotal length, and body weight than solitary colony founders. These studies did not investigate whether these physical parameters were correlated to genetic traits.

Genetic traits and partner selection

Phenotypic traits such as body weight and size can be predictors of the "genetic quality" of a mate. In long-term monogamous associations, such as those of termites, genetic quality of the mate and his/her offspring is predicted to be more

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valuable than simple offspring numbers (Brown 1997; Bonduriansky 2001). There are several theories concerning what genetic quality is and how it can be measured. According to Fisher (1915), partners should be chosen whose genes will enhance vigor and quality of offspring. This so-called "good genes" hypothesis is supported by both theoretical and empirical work (Petrie 1994). Brown (1997) proposed the "good-genes-as-heterozygosity hypothesis," that is, the good genes mates select for may actually be individual heterozygosity, because heterozygotes sire offspring with increased fitness. Heterozygotes are considered superior (Brown 1999) because of the benefits of increased genetic diversity (greater disease resistance), overdominance (recessive deleterious genes are not expressed in heterozygotes), and associative overdominance (direct effects of heterozygote alloenzymes or genes linked with them). Empirical studies have found correlations in various organisms between the degree of heterozygosity and the fitness-related traits, such as growth, viability, physiological performance, and bilateral symmetry (Mitton 1993; Brown 1999). Thus, the degree of heterozygosity might influence competitive ability, lifetime reproductive success, and partner preference (e.g., Höglund et al. 2002 in black grouse and Slate et al. 2000 in red deer). Individual heterozygosity is often interpreted as a genome-wide measure of heterozygosity and is expected to be inversely correlated with the degree of inbreeding and genetic relatedness of the parents (Mitton 1993, 1994). Inbred individuals (born to closely related parents) show reduced fitness, which is known as inbreeding depression (Blouin SF and Blouin M 1988). The genetic similarity of parents may influence many fitness components: including birth weight, survival, parasite load, and adult reproduction in some mammal and bird species (Amos et al. 2001).

Costs of inbreeding in termites

Models of the evolution of termite breeding systems generally have concluded that costs of inbreeding (mating between close relatives) are negligible or have only a mildly deleterious influence (Hamilton 1972; Bartz 1979; Roisin 1999) because for numerous termite species the colony life cycle includes inbreeding among neotenic offspring (derived from immature nymphs) after the death of the original colony founders. Moreover, high levels of inbreeding (believed to enhance kin selection) and the occurrence of inbreeding/outbreeding cycles (suggested to create relatedness asymmetries analogous to the social haplodiploid hymenoptera, i.e., some bees, wasps, and ants) were used to explain eusociality in diploid termites (Hamilton 1972; Bartz 1979; for criticism, see Husseneder et al. 1999).

However, empirical findings indicate varying costs of inbreeding among the Isoptera. For Termopsidae, inbreeding appears to have advantages. For *Zootermopsis angusticollis*, nest mate founder pairs suffered less mortality due to pathogens than non-nest mate pairs (Rosengaus and Traniello 1993; Calleri et al. 2005). The authors hypothesized that outbreeding depression, caused by the disruption of local adaptations or coadapted gene complexes, can cause a decrease in fitness; unrelated non-nest mate partners may introduce each other to novel pathogens (Rosengaus and Traniello 1993).

In contrast, recent studies found indications of inbreeding depression in subterranean termites. Husseneder et al. (2005) reported that workers of inbred colonies of the Formosan subterranean termite, *Coptotermes formosanus*, were smaller than those of their outbred counterparts, which might be an indication of stunted growth through inbreeding depression such as has been found in social spiders and crickets (Bilde et al. 2005; Rantala and Roff 2005). Fei and Henderson (2003) found lower long-term fecundity in later stages of colonies of *C. formosanus* raised from sibling pairs, although this deleterious effect of inbreeding was balanced by a higher initial survival of inbred colonies. DeHeer and Vargo (2006) reported a higher proportion of sibling pairs in precopulatory tandem pairs than in mature colonies of *Reticulitermes flavipes*. These data suggest that inbreeding depression may reduce the number of colonies raised by siblings over time. Given the evidence for subterranean termites thus far, it would seem that inbreeding depression may pose a significant factor affecting colony founding and early colony growth in this group. Therefore, mechanisms would be expected to exist that limit inbreeding by increasing the likelihood of pairing of unrelated alates after swarming.

Kin recognition and kin discrimination based on familiarity, phenotypic matching, or other factors correlated to the genetic makeup of an individual have been frequently postulated as key mechanisms of partner selection in many species (Blouin SF and Blouin M 1988; Pusey and Wolf 1996; Pillay 2002). Although the role of kin-biased behavior in colony mate discrimination and foraging in termites has received some attention (Adams 1991; Kaib et al. 1996; Husseneder et al. 1998, 2005), data on the involvement of kin recognition in mate selection of termites are rare. To our knowledge, Shellman-Reeve (2001) provided the only direct evidence of kin-biased mate choice of termites in nature; the author found that alates of the species *Z. nevadensis* avoid forming partnerships with close relatives.

Sensitive molecular markers, such as microsatellites, have been successfully employed to determine the degrees of relatedness, heterozygosity, and inbreeding in many species, including termites (e.g., Husseneder and Grace 2001a, 2001b; Goodisman and Crozier 2003; Husseneder et al. 2003, 2005; Vargo 2003a, 2003b; Vargo, Husseneder, and Grace 2003; Vargo, Husseneder, Grace, and Ring 2003; DeHeer and Vargo 2004; Dronnet et al. 2005; Hacker et al. 2005). Thus, microsatellite genotyping can be used to detect partner selection according to genetic factors.

Natural history and colony foundation of the Formosan subterranean termite

In this study, we focused on pair formation in the Formosan subterranean termite, *C. formosanus* Shiraki. A presumed native of China (Kistner 1985), this termite has become established in many countries and, since the end of the Second World War, has spread to at least 11 states in the United States (Woodson et al. 2001). Basic data regarding the breeding system and swarming behavior of this termite are available to support this study of partner selection (Husseneder et al. 2005, 2006; Vargo et al. 2006). Colonies are founded by a monogamous pair of reproductives, which stay together lifelong and produce millions of offspring (Shellman-Reeve 1999; Vargo, Husseneder, and Grace 2003; Vargo, Husseneder, Grace, and Ring 2003; Husseneder et al. 2005; Vargo et al. 2006). Later in the colony cycle, the original founder pair is replaced by multiple kings and queens, which are always the immature (neotenic) descendants of the single founder pair (Vargo, Husseneder, and Grace 2003; Vargo, Husseneder, Grace, and Ring 2003; Husseneder et al. 2005; Vargo et al. 2006). In most cases, the founding pair originates from alates, that is, the winged adults, which are produced by the colony during swarming season. In New Orleans, Louisiana, where the Formosan subterranean termite occurs in high population densities, alates swarm between late April and June with a peak in mid-May (King and Spink 1974). During this time, mass swarms tend to be synchronized among many colonies (Jones et al. 1988) but can also occur interspersed with localized small swarms (Henderson and Delaplane 1994). Colonies can produce thousands of alates in a single swarm event (Su and Scheffrahn 1987). Alates swarm shortly after sunset (King and Spink 1974) and are most visible in urban areas because they gather in large swarm aggregations surrounding light sources. After the flight, which lasts about 20–30 min, alates drop to the ground, shed their wings, and locate partners (Raina et al. 2003; Park et al. 2004).

Partner location, in certain termite species, is facilitated by "calling behavior," that is, the dealated female waives her abdomen emitting a male-attracting pheromone (Park et al. 2004). However, females of *C. formosanus* neither show calling behavior nor produce a male-attracting pheromone (Raina et al. 2003). Dealated termites form tandem pairs with the female in the lead. The male strives to maintain constant contact, touching the posterior region of the female's abdomen frequently with his antennae (Raina et al. 2003). Some tandems break up after a brief contact, but partner switching is rarely observed after a tandem has stayed together for 30 s or longer (similar to the related rhinotermitid *R. flavipes*, DeHeer and Vargo 2006). As soon as access to a suitable nest site is found, the tandem pairs move underground to mate. If successful, the tandem pair will be founders of a new colony. New colonies develop for 5–8 years before reaching maturity, which is marked by the production of alates (Huang and Chen 1984).

Objective

The objective of this study was to evaluate whether tandem pairs of *C. formosanus* form randomly after swarms or if phenotypic or genetic characteristics of swarming adults play a role in the formation of tandem pairs. Weights and head widths were compared between adults in tandem pairs and individuals of both sexes that did not engage in tandem running to establish whether larger size increases the likelihood of being in a tandem versus being single. The null hypothesis was that members of tandem pairs and single individuals do not differ in their weights and head widths. Weights and head widths were tested for correlation between tandem partners to establish whether large termites form tandems with a large partner. The null hypothesis was that size parameters are not correlated between tandem partners.

Pairwise relatedness values were tested for significant differences between tandem partners and termites from different tandem pairs in each of 5 light trap samples. The null hypothesis was that there are no differences in relatedness between tandem pairs and relatedness to members of other tandem pairs, that is, no partner choice based on relatedness is detectable. The degree of individual heterozygosity was compared for both sexes engaged in tandem pairs or staying single. The null hypothesis was that neither males nor females have significantly different degrees of heterozygosity, regardless of whether they are engaged in tandem pairs or if they stayed single. Degrees of heterozygosity of tandem partners were correlated to identify the possible influence of heterozygosity on partner choice.

Mate selection theories predict correlations between the degree of heterozygosity and phenotypic characteristics, which would allow individuals to use phenotypic traits as proximate cues to ultimately assess inbreeding of a potential partner. Also, a negative correlation of heterozygosity with body size and weight could indicate inbreeding depression. Therefore, we tested if these phenotypic traits are correlated to the degree of heterozygosity.

MATERIALS AND METHODS

Alate collection and formation of tandem pairs

During a mass swarming event (3 June 2003), live alates of the Formosan subterranean termite were collected using light traps. The traps were buckets with a battery-operated black light attached to attract the alates. A total of 34 light traps were dispersed at street corners of the French Quarter, New Orleans, LA. The number of alates in each trap was determined (range of 11–4492). Alates from 5 light traps, separated from each other by 145–1014 m (identification numbers: 5, 16, 17, 24, and 33, map in Husseneder et al. 2006), were immediately transported to the laboratory. There, random samples of alates representing the composition of males and females from each of the 5 traps were put into 5 separate arenas (size 60 × 60 cm).

In the arenas, alates dropped their wings and began to form tandem pairs. Tandem pairs were sampled during a period of 30 min, which is approximately the time frame it takes in nature for all tandem pairs of a swarm to form and disappear into the ground. From each arena containing the catch of 1 of 5 light traps, 20 tandem running pairs (all dealated and running in tandem for 30 s or longer) were collected in separate vials and preserved in 100% ethanol. All single termites left in the arena at the end of the trial period were also preserved in alcohol. Because the trial period was only 30 min, minimal mortality was observed. The total number of alates collected for the analyses (97–151) and the ratios of males and females in these random samples are presented in Table 1.

Sex ratio

For each sample, the sex of termites in tandem pairs and of the remaining single termites was determined by examining the terminal abdominal sterna as described in Higa (1981)

Table 1

Number of heterosexual (male/female [m/f]) and homosexual tandem pairs (male–male and female–female), male:female ratio (*n* = number of alates without the 20 tandem pairs), deviation from equal sex ratio in light trap samples, deviation of sex ratios of single versus tandem-forming adults, and deviation from random pairing based on the sex ratio in light trap samples

Trap ID no.	No. of tandem pairs m/f	m/m	f/f	m/f Ratio (*n*)	Deviation from 1:1	Deviation of sex ratios of singles versus tandem pairs	Deviation from random pairing
5	18	2	0	1.27 (100)	ns	ns	$P = 0.01$
16	19	1	0	1.04 (57)	ns	ns	$P < 0.001$
17	8	0	12	0.71 (77)	ns	$P = 0.02$	ns
24	19	0	1	0.85 (111)	ns	ns	$P < 0.001$
33	18	0	2	1.73 (82)	$P = 0.02$	ns	$P < 0.001$

ns, not significant.

using a stereomicroscope (LEICA MZ16, Meyers Instruments, Houston, TX). Sex ratios in each sample were tested for significant deviation from 1:1 using binomial tests and Fisher's exact test (SPSS 11.5 for Windows, SPSS Inc., Chicago, IL). The number of same sex and opposite sex tandem pairs was determined in each sample, and deviations from random formation of tandem pairs were tested using chi-square tests.

Phenotypic traits

Weights of individuals were measured using a balance (Mettler-Toledo, Inc., Columbus, OH) after letting the alcohol evaporate for a few minutes until the weight of the sample was stable. Head sizes were measured as the distance between the inner edge of the compound eyes with a micrometer inserted into the ocular of the stereoscope (×50 magnification); the ocular micrometer was calibrated using a stage micrometer (1 unit = 0.028 mm).

Weights and head sizes were compared between adults in tandem pairs and single individuals of both sexes using nonparametric tests (2-sided Mann–Whitney *U*-test; SPSS) to establish whether larger size increases the likelihood of being in a tandem (vs. being single). Weights and head size were also correlated between tandem partners to establish whether larger termites form tandems with a larger partner (Pearson's correlation).

Genetic traits

Microsatellite genotyping was used to determine degrees of pairwise relatedness of both individuals in tandem pairs and those staying single and to determine each individual's degree of heterozygosity. Microsatellite genotyping is a highly sensitive molecular genetic technique. This method detects genetic variance of simple, repeated tandem sequences, 1–6 bp in length, dispersed in noncoding regions across the eukaryotic genome. These markers are highly polymorphic due to the variable number of tandem sequence repeats. The different alleles are inherited in simple Mendelian fashion and are likely selectively neutral. Microsatellite markers for *C. formosanus* were developed by Vargo and Henderson (2000).

After measurements of physical characteristics were completed, DNA was extracted using the DNeasy Tissue Kit (Qiagen Inc., Valencia, CA) from the members of 20 tandem pairs collected from each of the 5 light trap samples as well as from 20 males and 20 females that did not engage in tandem running. Individuals were scored at the same 8 microsatellite loci previously used to describe the genetic structure of colonies and alate samples of Formosan subterranean termites in New Orleans (Husseneder et al. 2005, 2006). A detailed description of the conditions for polymerase chain reaction and genotype scoring procedures can be found in Vargo and Henderson (2000).

From the genotypic frequencies at the 8 microsatellite loci, pairwise relatedness coefficients between individuals were calculated with the software SpaGeDi (Hardy and Vekemans 2002). Pairwise relatedness values were tested for significant differences between tandem partners and termites from different tandem pairs in each sample (Kruskal–Wallis analysis of variance [ANOVA], SPSS). Tandem partners and single males and females were tested for genotypic differences by comparing degrees of relatedness (r), inbreeding (F_{IS}), and observed heterozygosity (Ho) using permutation tests (FSTAT).

The individual degree of inbreeding for tandem partners and single termites was measured using individual heterozygosity (measured as the proportion of loci for which an individual was heterozygous, Coltman et al. 1998). In addition, heterozygosity was measured using the microsatellite-specific parameter d^2 (Coltman et al. 1999). This parameter uses the stepwise mutation model of microsatellite alleles and is based on the measure of population differentiation of Goldstein et al. (1995). The value of d^2 is calculated as the squared difference in repeat units between 2 alleles at each locus averaged over all loci (Coulson et al. 1998, 1999; Höglund et al. 2002). The degree of heterozygosity was compared for males and females engaged in tandem pairs and males and females that remained single using the nonparametric Mann–Whitney *U*-test (SPSS) to establish whether heterozygosity influences the likelihood of being engaged in tandem running. Also, degrees of heterozygosity of tandem partners were tested for correlation to identify the possible influence of the degree of heterozygosity on competitive advantage or partner choice. To test for inbreeding depression, degrees of heterozygosity were tested for correlation with individual body weight and head width.

RESULTS

The male to female ratio in 4 of the 5 samples did not significantly deviate from 1:1 ($P > 0.20$, binomial test, Table 1). Significant male bias ($P = 0.02$, binomial test) was found in only one sample. When alates from all 5 samples were combined, the sex ratio was equal ($P > 0.20$, binomial test).

In 4 of the 5 samples, the sex ratio in tandem pairs calculated from the number of male–female tandem pairs, male–male tandem pairs, and female–female tandem pairs in each sample did not deviate from the sex ratio of all alates in the respective sample ($P > 0.20$ for the samples from traps 5, 16, 24, and 33; $P = 0.02$ for the sample from trap 17; Fisher's exact test; Table 1). Thus, in the majority of light trap samples, tandem pairs were a representative sample of the alate population caught in the trap, that is, both males and females were equally "ready" to engage in tandem running. The majority of alates formed tandem pairs with the opposite sex rather than pairing randomly ($P \leq 0.01$; degrees of freedom [df] = 2, chi square ranging from 14.4 to 18.1). Only alates from the most female-biased sample (trap 17) showed marginal prevalence of female–female tandem pairs ($P = 0.07$, df = 2, chi square = 5.4; Table 1).

The measurements of head widths and body weights of males and females, both engaged in tandem running and single, are presented in Table 2. Females were larger and heavier than males ($P < 0.01$, 2-tailed Mann–Whitney *U*-test). Overall, female head widths ranged from 1.13 to 1.48 mm (mean = 1.29 mm, standard deviation [SD] = 0.06, $n = 212$) compared with male head widths (1.13–1.43 mm, mean = 1.26 mm, SD = 0.06, $n = 188$). Female body weights ranged from 4.3 to 10.3 mg (mean = 6.9 mg, SD =1.2, $n = 212$), and male weights ranged from 2.9 to 8.3 mg (mean = 5.3 mg, SD = 1.0, $n = 188$).

Males collected from heterosexual tandem pairs had larger heads than males that remained single ($P < 0.001$, $n = 82$ in tandem pairs, $n = 100$ single, 2-tailed Mann–Whitney *U*-test). Testing each light trap sample separately revealed significant differences in 3 samples ($P < 0.001$ in traps 5 and 16; $P = 0.011$ in trap 24, 2-tailed Mann–Whitney *U*-test; Table 2). No difference was found between members of tandem pairs and single termites for female head sizes and weights and male weights ($P > 0.20$, 2-tailed Mann–Whitney *U*-test).

Females in heterosexual tandems had significantly higher weights than females in homosexual tandems (6.92 mg, SD = 0.98, $n = 82$ vs. 6.40 mg, SD = 0.90, $n = 30$, $P = 0.01$) and marginally larger heads (1.30 mm, SD = 0.07, $n = 82$ vs. 1.27 mm, SD = 0.05, $n = 30$, $P = 0.09$; Mann–Whitney *U*-test). Tests did not reveal significant differences between males in homosexual tandems and males in heterosexual tandems in weight (5.20 mg, SD = 1.2, $n = 6$ vs. 5.35 mg, SD = 0.88, $n = 82$,

Table 2

Comparison of average head widths (distance between compound eyes) and weights of males (m) and females (f) collected as singles or from male–female tandem pairs in 5 light trap samples (ID numbers: 5, 16, 17, 24, and 33)

	Head (mm)				Weight (mg)			
	Single		Tandem		Single		Tandem	
	m	f	m	f	m	f	m	f
5	1.21[a] SD = 0.04	1.32 SD = 0.04	1.29[a] SD = 0.06	1.31 SD = 0.06	5.6 SD = 1.1	7.6 SD = 1.1	5.6 SD = 1.9	7.4 SD = 1.2
16	1.24[a] SD = 0.06	1.29 SD = 0.05	1.29[a] SD = 0.05	1.31 SD = 0.05	4.8 SD = 1.0	6.2 SD = 0.9	4.6 SD = 1.9	5.5 SD = 1.9
17	1.25 SD = 0.05	1.32 SD = 0.06	1.27 SD = 0.07	1.29 SD = 0.04	5.6 SD = 0.9	6.5 SD = 1.1	5.6 SD = 1.4	6.5 SD = 1.2
24	1.26[a] SD = 0.06	1.28 SD = 0.05	1.32[a] SD = 0.07	1.29 SD = 0.06	5.7 SD = 1.0	7.3 SD = 1.2	7.2 SD = 1.0	7.0 SD = 1.6
33	1.24 SD = 0.04	1.30 SD = 0.06	1.24 SD = 0.07	1.26 SD = 0.06	4.7 SD = 0.7	6.3 SD = 0.7	4.3 SD = 0.7	6.1 SD = 1.1

[a] Significant difference between head widths of single males and males collected from male–female tandem pairs (2-tailed Mann–Whitney *U*-test, 5% significance level).

$P > 0.20$) and head width (1.3 mm, SD = 0.03, $n = 6$ vs. 1.28, SD = 0.07, $n = 82$, $P > 0.20$; Mann–Whitney *U*-test), probably due to the small sample size.

Head widths of individuals collected from heterosexual tandem pairs were not significantly correlated to their weights in most of the light trap samples (exceptions were as follows: trap 5, females: $r = 0.80$, $P < 0.01$, $n = 18$; trap 24, males: $r = 0.62$, $P < 0.01$, $n = 19$). When sample size was increased by combining all individuals collected from heterosexual tandem pairs across all 5 samples, head widths and weights were significantly correlated (males: $r = 0.66$, $P < 0.01$; females: $r = 0.51$, $P < 0.01$, $n = 82$).

Average body weights of females in heterosexual tandem pairs varied across samples ($P = 0.02$); however, the weights of males was not significantly different among samples ($P = 0.14$, $n = 82$, df = 4, Kruskal–Wallis ANOVA). Average head widths of males running in tandem with females varied across samples ($P = 0.03$); however, head widths of their female tandem partners did not differ among samples ($P > 0.20$, $n = 82$, df = 4, Kruskal–Wallis ANOVA). Analyzing the samples separately revealed significant positive correlations between weights of male and female tandem partners in 2 samples (trap 5: $r = 0.66$, $P = 0.003$, $n = 18$; trap 24: $r = 0.53$, $P = 0.019$, $n = 19$) and between head widths of tandem partners in 3 samples (trap 5: $r = 0.56$, $P = 0.016$, $n = 18$; trap 16: $r = 0.63$, $P = 0.004$, $n = 19$; trap 24: $r = 0.57$, $P = 0.01$, $n = 19$). When heterosexual tandem pairs were combined across all samples, positive correlations were found between male and female weights ($r = 0.56$, $P = 0.008$, $n = 82$) and male and female head widths in tandem pairs ($r = 0.37$, $P < 0.001$, $n = 82$, Figure 1).

To test for kin-biased partner selection, we compared the pairwise relatedness of tandem partners with the pairwise relatedness between all termites that belonged to different tandem pairs in each sample (Table 3). Only in one sample (trap 33) were tandem pairs significantly more related to each other than to individuals from different tandem pairs ($P = 0.03$, df = 1, chi square = 4.9, Kruskal–Wallis ANOVA). In the remaining 4 samples, no significant difference in the pairwise degrees of relatedness was found (traps 5, 16, and 24: $P > 0.20$, df = 1, chi square = 0.2–0.8; trap 17: $P = 0.10$, df = 1, chi square = 3.6; Kruskal–Wallis ANOVA).

Whereas there was no consistent difference in relatedness between tandem partners and individuals collected from different tandem pairs, there was significant genotypic differentiation between termites which were not engaged in tandem running (singles) and members of tandem pairs (900

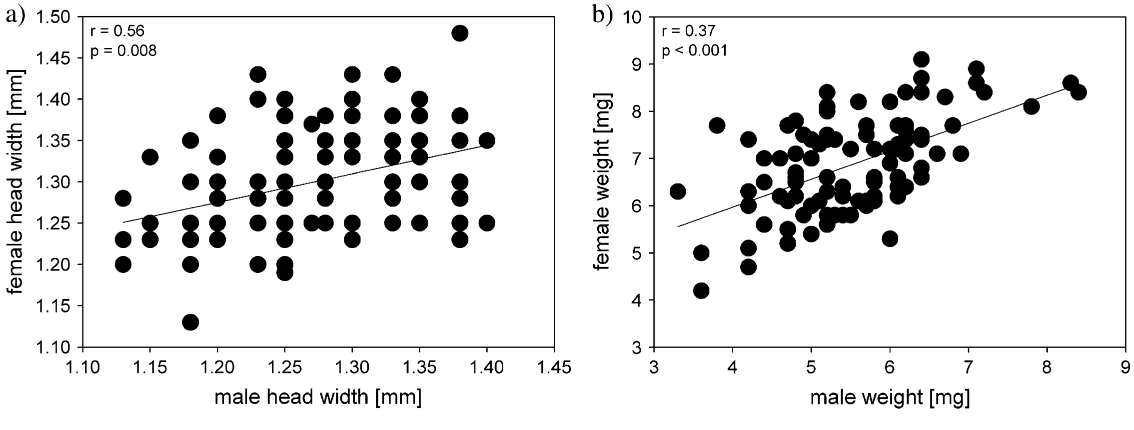

Figure 1
Correlations of head widths (distance between compound eyes) and weights between male and female tandem partners of the 5 light trap samples.

Table 3

Average pairwise relatedness between tandem partners and among termites collected from separate tandem pairs in 5 light trap samples

	Sample ID no.				
Relatedness	5	16	17	24	33[a]
Tandem partners	−0.08	0.06	−0.09	−0.01	0.12
SD	0.47	0.22	0.31	0.24	0.21
Tandem partners	−0.02	−0.03	−0.02	−0.03	−0.03
SD	0.43	0.31	0.27	0.28	0.26

[a] Significant difference between tandem partners and individuals collected from different tandem pairs (Kruskal–Wallis ANOVA, 5% significance level).

permutations, 5% level, FSTAT). When females and males were analyzed separately, no genotypic difference between male members of tandem pairs and single males was detected; however, genotypic differences were revealed between females collected from male–female tandem pairs and single females in 3 of the 5 light trap samples (traps 17, 24, and 33; 3800 permutations, 5% level, FSTAT). To characterize the genotypic differentiation further, we compared the degrees of relatedness (r), inbreeding (F_{IS}), and observed heterozygosity (Ho) of female members of tandem pairs and single females from all 5 samples. Average relatedness among single females ($r = 0.14$) was not significantly different from average relatedness among females from male–female tandem pairs ($r = 0.13$, 5000 permutations, FSTAT). However, the degree of inbreeding was marginally higher, and the observed heterozygosity was marginally lower in single females ($F_{\mathrm{IS}} = 0.28$, Ho = 0.44) than in female members of tandem pairs ($F_{\mathrm{IS}} = 0.15$, $P = 0.09$; Ho = 0.53, $P = 0.08$; 5000 permutations, FSTAT).

In 4 of the 5 samples, the individual heterozygosity (measured as the proportion of heterozygote loci) of females engaged in heterosexual tandem pairs was higher than the heterozygosity of single males and females and of males collected from tandem pairs; in about half the cases, this difference was significant at the 5% level (Table 4). Only the heterozygosity of males collected from tandem pairs differed significantly among samples ($P = 0.01$, df = 4, chi square = 13.3, Kruskal–Wallis ANOVA). Because the heterozygosity of single females and female members of tandem pairs did not differ significantly among samples ($P > 0.20$) and the heterozygosity of males collected from tandem pairs only differed marginally among samples ($P = 0.058$, df = 4, chi square = 9.1, Kruskal–Wallis ANOVA), the samples were combined to increase sample size.

Table 4

Mean individual heterozygosity of single and tandem running males (m) and females (f) in 5 light trap samples

	Single		Tandem	
	m	f	m	f
5	0.32^{a} SD = 0.16 n = 20	0.41^{a} SD = 0.16 n = 20	0.34^{a} SD = 0.20 n = 18	0.51^{b} SD = 0.17 n = 18
16	0.43^{ab} SD = 0.18 n = 20	0.46^{ab} SD = 0.17 n = 20	0.34^{a} SD = 0.17 n = 19	0.50^{b} SD = 0.17 n = 19
17	0.42^{a} SD = 0.17 n = 20	0.49^{ab} SD = 0.19 n = 20	0.43^{a} SD = 0.19 n = 8	0.56^{b} SD = 0.20 n = 8
24	0.56^{ab} SD = 0.22 n = 20	0.51^{a} SD = 0.17 n = 20	0.51^{a} SD = 0.16 n = 19	0.65^{b} SD = 0.16 n = 19
33	0.55^{a} SD = 0.16 n = 20	0.51^{a} SD = 0.20 n = 20	0.57^{a} SD = 0.22 n = 18	0.55^{a} SD = 0.20 n = 18

n, number of individuals. Significant differences within light trap samples (same row) are indicated by different letters (2-tailed Mann–Whitney U-test, 5% significance level).

Across all samples, the individual heterozygosity of females engaged in heterosexual tandem pairs was significantly higher than the heterozygosity of single males ($P = 0.001$), males collected from tandem pairs ($P = 0.001$), and single females ($P = 0.026$, 2-tailed Mann–Whitney U-tests). No significant differences were detected between the degrees of heterozygosity of single males versus single females, of single males versus males from tandem pairs, or of single females versus males from tandem pairs ($P > 0.20$, 2-tailed Mann–Whitney U-tests, Figure 2). The same pattern occurred when the degree of heterozygosity was measured by the microsatellite-specific parameter d^2, although with only marginal significance values ($P = 0.064$ for females collected from tandem pairs vs. both single males and single females, 2-tailed Mann–Whitney U-test).

Degrees of heterozygosity of tandem partners were not significantly correlated (Pearson's $r = -0.03$, $P > 0.20$ for proportions of heterozygote loci; Pearson's $r = 0.03$, $P > 0.20$ for d^2). Heterozygosity was not significantly correlated to head width ($P > 0.20$ for proportions of heterozygote loci and d^2) or weight of an individual ($P = 0.09$ for proportions of heterozygote loci and $P = 0.17$ for d^2).

DISCUSSION

In most termite species, including *C. formosanus*, colonies are founded by one king and one queen (Husseneder et al. 1999, 2005; Shellman-Reeve 1999; Thorne et al. 1999; Vargo, Husseneder, and Grace 2003; Vargo, Husseneder, Grace, and Ring 2003; Vargo et al. 2006). This monogamy, which is

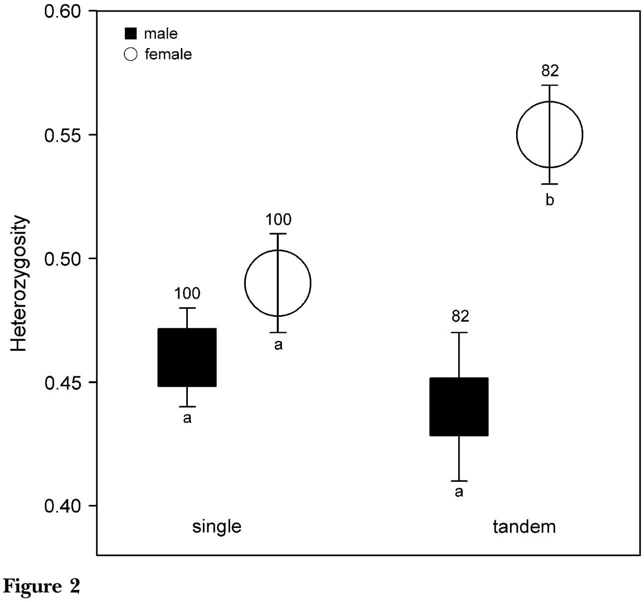

Figure 2

Heterozygosity of males and females engaged in tandem running and single males and females. Error bars indicate standard errors. Sample sizes are written above error bars. Different letters below error bars indicate significant difference (Mann–Whitney U-tests, 5% significance levels).

rarely found in insects, lasts for the lifetime of the founder pair. The founding partners depend on each other for raising the young (biparental care), and the parental investment during the first stages of colony development is considerable (Shellman-Reeve 2001). Because of this peculiar life cycle, both sexes are predicted to exhibit precopulatory selectiveness (Shellman-Reeve 2001). Consistent with these theoretical predictions, we found that size-related traits and heterozygosity play a role in pair formation in the Formosan subterranean termite.

Males collected from tandem pairs had larger heads than single males. This suggests that large males have a competitive advantage and/or that females choose to form tandems with large males. In addition, head width of tandem partners and body weight of tandem partners were correlated, that is, males with large heads paired with females with large heads and heavy males paired with heavy females. These phenotypic trait correlations are not merely artifacts of limited sampling of alates from 1 or 2 colonies per light trap because termites in each light trap sample originated from an average of 13 colonies (Husseneder et al. 2006). Because alates did not prefer to form tandem pairs with nest mates in this study, the correlations are not explained by size variation among colonies. Also, the correlations were not just an artifact caused by size differences of both males and females from different light trap samples because weights of males and head widths of females were not significantly different across samples.

Pairing advantage and mate choice based on size-related characters are common in insects. Size and weight are phenotypic traits often related to fecundity and health (correlates of good genes, Bonduriansky 2001). In termites, partner selection based on size is supported by findings of Shellman-Reeve (1999), who observed that females of *Z. nevadensis* choose males with big heads, whereas males choose females according to body mass and lipid mass. The author attributed this to the advantage of having large fat reserves when starting a new colony. Matsuura and Nishida (2001) reported that males of a Japanese subterranean termite, *R. speratus*, paired with heavier females; females apparently gain fitness advantage from pairing with heavier males, because they gain more weight if they pair with large males. Kitade et al. (2004) showed that paired dealates in incipient colonies of *R. kanmonensis* tended to be larger in head width and male pronotal length than solitary reproductives, which suggests sexual selection for body size (via intrasexual competition or mate choice) in both sexes. As in our study, body weight was correlated between paired males and females, but no such correlation was found in head width or pronotal length (Kitade et al. 2004). It may be argued that these phenotypic correlations are due to tandem pair–forming adults sharing similarity in ecological habitat, as has been proposed for phenotypic correlations between incipient colony reproductives (Shellman-Reeve 1994; Kitade et al. 2004). However, tandem partners from our study likely came from different colonies, and thus different ecological environments. Hence, pairing advantage of large individuals rather than individuals from common environments must explain the phenotypic trait correlations.

Mechanisms that limit inbreeding are expected to exist in subterranean termites because evidence for inbreeding depression is mounting, such as higher mortality in inbred incipient colonies in *R. flavipes* (DeHeer and Vargo 2006) and lower lifetime fecundity in inbred *C. formosanus* colonies (Fei and Henderson 2003). From the point of colony foundation, inbreeding can only increase within colonies due to the inbreeding cycles of generations of neotenic reproductives (offspring of the colony) that propagate the colony after the death of the founders. Outbreeding can only be promoted during swarming and tandem running because there is no evidence of adoption of unrelated reproductives in *C. formosanus* and because colony fusion is a rare event if it occurs at all (Vargo, Husseneder, and Grace 2003; Vargo, Husseneder, Grace, and Ring 2003; Husseneder et al. 2005; Vargo et al. 2006). Outbreeding is promoted either by selecting unrelated partners during tandem running involving nest mate or kin recognition and discrimination or by dispersal mechanisms that increase the likelihood of unrelated alates to meet (Husseneder et al. 2006).

Kin recognition and kin discrimination have been frequently postulated to play a key role in partner selection (Blouin SF and Blouin M 1988; Pusey and Wolf 1996; Pillay 2002). In most insect species investigated, mate choice is random with respect to kinship (Waldman 1988). However, in termites, it has been shown that alates of a termite species (*Z. nevadensis*) avoided mating with close relatives (Shellman-Reeve 2001).

Mean relatedness between tandem partners of *C. formosanus* was not significantly different from the mean relatedness between termites belonging to different tandem pairs. Thus, kin recognition and discrimination did not play a key role in the choice of tandem partners in the Formosan subterranean termite. Similarly, the distribution of mitochondrial haplotypes of colony founders in a Japanese subterranean termite (*R. kanmonenis*, Kitade et al. 2004) suggested random mating.

In a previous study, other mechanisms were detected that increased the likelihood of pairing of unrelated alates in *C. formosanus* and thus limited inbreeding (Husseneder et al. 2006). Sufficient swarming distance and the synchronization of mass swarm events (Henderson and Delaplane 1994; Henderson 1996) ensure mixing of alates from multiple different colonies resulting in high genetic diversity within each light trap sample (Husseneder et al. 2006). Genotypic differentiation between males and females, which was caused by sex-biased investment at the colony level rather than sex-biased dispersal, contributed to reduce the incidence of sibling pairings (Husseneder et al. 2006).

Pairing between largely unrelated adults (due to dispersal rather than kin recognition) decreases the degree of inbreeding and thus leads to increased heterozygosity in the offspring. Heterosis (heterozygote advantage) is documented in many vertebrate and invertebrate species (Tregenza and Wedell 2000). For example, color pattern of the heterozygous morph of the African butterfly *Danaus chrysippus* increased the mating advantage of males (Smith 1981). Similarly, males of 2 species of sulphur butterflies that were heterozygous at 3 alloenzyme loci had mating advantage (Watt et al. 1986). Early work on *Drosophila* spp. revealed that flies heterozygous for certain inversions showed superior fitness and developmental homoeostasis compared with their homozygote counterparts (Dobzhansky and Wallace 1953; Spiess and Langer 1966), which was correlated to increased courting and mating frequency of heterozygote males (Brncic and Koref-Santibanez 1964). Also, heterozygous brine shrimp had mating advantage (Mitton 1997).

In our study of the termite *C. formosanus*, females with a higher degree of heterozygosity were more likely to be engaged in tandem pairs than females or males with lower degrees of heterozygosity. Yet, no correlation was detected between the degrees of heterozygosity of tandem partners, indicating that heterozygosity does not influence mutual partner selection. The higher degree of heterozygosity in females collected from tandem pairs is therefore likely due to competitive advantage of heterozygote females and/or males choosing heterozygote females, but not vice versa. Heterozygosity was not correlated to size-related phenotypic parameters like head width and weight of an individual in this study. Thus, size alone may not be a useful indicator to assess degree of

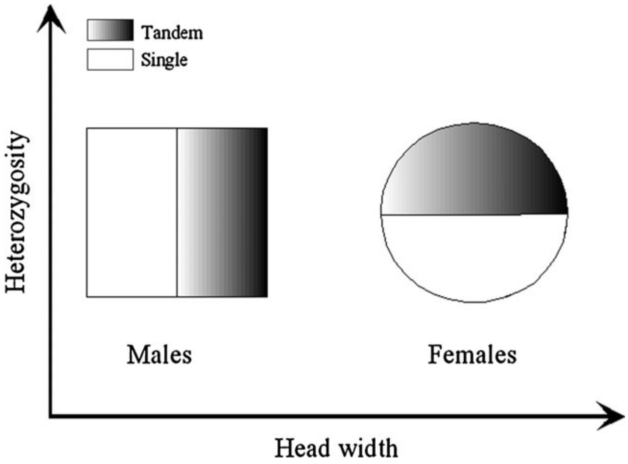

Figure 3
Influence of head width and heterozygosity on the likelihood of forming tandem pairs. Males with large heads and females with high heterozygosity are more likely to be found in tandem pairs (shaded vs. white areas). Males with large heads form tandems preferably with big-headed females (intensity of shading).

heterozygosity. This was surprising because pairing advantage in females was linked not only to heterozygosity but also to size. The lack of correlation suggests that size and heterozygosity could act independently. For example, males could prefer heterozygous females, but at the same time, large females could have a competitive advantage over smaller females when tandem pairs are formed.

Because it is unlikely that pair-forming adults recognize heterozygosity directly and because size is not a reliable predictor for heterozygosity, the proximate cues determining pairing advantage remain unknown. We are currently investigating the effect of inbreeding on fluctuating asymmetry and symbiont diversity, which might also play a role in mate choice and/or competitive advantage.

The fact that females with a higher degree of heterozygosity have an advantage in forming tandem pairs and thus, in all likelihood, a mating advantage leads to the following prediction: colonies producing mainly heterozygous offspring (i.e., colonies headed by unrelated parents) should produce mainly female alates, whereas colonies headed by inbred, related parents (neotenics) should produce more males. Evidence supporting this prediction has been found in several species within the genus *Coptotermes*, where sex-biased alate production depended on the breeding system of the colony. In *C. formosanus*, male alates had significantly higher inbreeding levels than females. The degrees of inbreeding suggested that males originated from inbred extended family colonies headed by neotenics, whereas females were more likely to be produced by outbred simple family colonies (Husseneder et al. 2006). Similarly, orphaned colonies headed by neotenics in *Coptotermes lacteus* produced almost exclusively males (Lenz and Runko 1993; Roisin and Lenz 2002). Roisin and Lenz (2002) suggested similar characteristics in *Coptotermes acinaciformis*.

In summary, a combination of size-related physical traits and inbreeding related genetic traits influence sexual selection and/or competitive advantage of males and females in the Formosan subterranean termite. Males with increased head width and females with increased heterozygosity are more likely to be engaged in tandem pairs. Large males (with increased head width and weight) preferably form tandems with large females (Figure 3).

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REFERENCES

Adams ES. 1991. Nest-mate recognition based on heritable odors in the termite *Microcerotermes argorerus*. Proc Natl Acad Sci USA. 88: 2031–2034.

Alexander RD, Marshall DC, Cooley JR. 1997. Evolutionary perspectives on insect mating. In: Choe JC, Crespi BJ, editors. The evolution of mating systems in insects and arachnids. Cambridge (MA): Cambridge University Press. p. 4–31.

Amos W, Wilmer JW, Fullard K, Burg TM, Croxall JP, Bloch D, Coulson T. 2001. The influence of parental relatedness on reproductive success. Proc R Soc Lond B Biol Sci. 268:2021–2027.

Bartz SH. 1979. Evolution of eusociality in termites. Proc Natl Acad Sci USA. 76:5764–5768.

Bilde TY, Lubin D, Smith J, Schneider M, Maklakov AA. 2005. The transition to social inbred mating systems in spiders: role of inbreeding tolerance in a subsocial predecessor. Evolution. 59: 160–174.

Blouin SF, Blouin M. 1988. Inbreeding avoidance behaviors. Trends Ecol Evol. 3:230–233.

Bonduriansky R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. Biol Rev. 76:305–339.

Brncic D, Koref-Santibanez S. 1964. Rate of development and inversion polymorphism in *Drosophila pavani*. Genetics. 49:585–591.

Brown JL. 1997. A theory of mate choice based on heterozygosity. Behav Ecol. 8:60–65.

Brown JL. 1999. The new heterozygosity theory of mate choice and the MHC. Genetica. 104:215–221.

Calleri DV, Rosengaus RB, Traniello JFA. 2005. Disease and colony foundation in the dampwood termite *Zootermopsis angusticollis*: the survival advantage of nestmate pairs. Naturwissenschaften. 92: 300–304.

Coltman DW, Bowen WD, Wright JM. 1998. Birth weight and neonatal survival of harbour seal pups are positively correlated with genetic variation measured by microsatellites. Proc R Soc Lond B Biol Sci. 265:803–809.

Coltman DW, Pilkington JG, Smith JA, Pemberton JM. 1999. Parasite-mediated selection against inbred soya sheep in a free-living, island population. Evolution. 53:1259–1267.

Coulson T, Albon S, Slate J, Pemberton J. 1999. Microsatellite loci reveal sex-dependent responses to inbreeding and outbreeding in red deer calves. Evolution. 53:1951–1960.

Coulson TN, Pemberton JM, Albon SD, Beaumont M, Marshall TC, Slate J, Guiness FE, Clutton-Brock TH. 1998. Microsatellites reveal heterosis in red deer. Proc R Soc Lond B Biol Sci. 265:489–495.

DeHeer CJ, Vargo EL. 2004. Colony genetic organization and colony fusion in the termite *Reticulitermes flavipes* as revealed by foraging patterns over time and space. Mol Ecol. 13:431–441.

DeHeer CJ, Vargo EL. 2006. An indirect test of inbreeding depression in the termites *Reticulitermes flavipes* and *Reticulitermes virginicus*. Behav Ecol Sociobiol. 59:753–761.

Dobzhansky T, Wallace B. 1953. The genetics of homeostasis in *Drosophila*. Proc Natl Acad Sci USA. 39:162–171.

Dronnet S, Chapuisat M, Vargo EL, Lohou C, Bagnères A-G. 2005. Genetic analysis of the breeding system of an invasive subterranean termite, *Reticulitermes santonensis*, in urban and natural habitats. Mol Ecol. 14:1311–1320.

Fei HX, Henderson G. 2003. Comparative study of incipient colony development in the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera, Rhinotermitidae). Insectes Soc. 50: 226–233.

Fisher RA. 1915. The evolution of sexual preference. Eugen rev. 7: 184–192.

Goldstein D, Linares AR, Cavallisforza LL, Feldman MW. 1995. An evaluation of genetic distances for use with microsatellite loci. Genetics. 139:463–471.

Goodisman MAD, Crozier RH. 2003. Association between caste and genotype in the termite *Mastotermes darwiniensis* Froggatt (Isoptera: Mastotermitidae). Aust J Entomol. 42:1–5.

Hacker M, Kaib M, Bagine RKN, Epplen JT, Brandl R. 2005. Unrelated queens coexist in colonies of the termite *Macrotermes michaelseni.* Mol Ecol. 14:1527–1532.

Hamilton WD. 1972. Altruism and related phenomena, mainly in social insects. Annu Rev Ecol Syst. 3:193–232.

Hardy OJ, Vekemans X. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes. 2:618–620.

Henderson G. 1996. Alate production, flight phenology, and sex-ratio in *Coptotermes formosanus* Shiraki, an introduced subterranean termite in New Orleans, Louisiana. Sociobiology. 28:319–326.

Henderson G, Delaplane KS. 1994. Formosan subterranean termite swarming behavior and alate sex-ratio (Isoptera, Rhinotermitidae). Insectes Soc. 41:19–28.

Higa SY. 1981. Flight, colony foundation, and development of the gonads of the primary reproductives of the Formosan subterranean termite. *Coptotermes formosanus* Shiraki [PhD dissertation]. [Honolulu (HI)]: University of Hawaii. 173 p.

Höglund J, Piertney SB, Alatalo RV, Lindell J, Lundberg A, Rintamäki PT. 2002. Inbreeding depression and male fitness in black grouse. Proc R Soc Lond B Biol Sci. 269:711–715.

Huang LW, Chen LL. 1984. Biology and colony development of *Coptotermes formosanus* Shiraki. Acta Entomol Sin. 27:64–69.

Husseneder C, Grace JK. 2001a. Evaluation of DNA fingerprinting, aggression tests, and morphometry as tools for colony delineation of the Formosan subterranean termite. J Insect Behav. 14:173–186.

Husseneder C, Grace JK. 2001b. Similarity is relative: hierarchy of genetic similarities in the Formosan subterranean termite (Isoptera: Rhinotermitidae) in Hawaii. Environ Entomol. 30:262–266.

Husseneder C, Grace JK, Messenger MT, Vargo EL, Su N-Y. 2003. Describing the spatial and social organization of Formosan subterranean termite colonies in Armstrong Park, New Orleans. Sociobiology. 41:61–65.

Husseneder C, Kaib M, Epplen C, Epplen JT, Brandl R. 1998. Variation between and within colonies in the termite: morphology, genomic DNA, and behaviour. Mol Ecol. 7:983–990.

Husseneder C, Kaib M, Epplen C, Epplen JT, Brandl R. 1999. Within-colony relatedness in a termite species: genetic roads to eusociality? Behaviour. 136:1045–1063.

Husseneder C, Messenger MT, Su N-Y, Grace JK, Vargo EL. 2005. Colony social organization and population genetic structure of an introduced population of the Formosan subterranean termite from New Orleans, Louisiana. U.S.A. J Econ Entomol. 98:1421–1434.

Husseneder C, Simms DM, Ring DR. 2006. Genetic diversity and genotypic differentiation between the sexes in swarm aggregations decrease inbreeding in the Formosan subterranean termite. Insect Soc. 53:212–219.

Jones SC, La Fage JP, Howard RW. 1988. Isopteran sex-ratios: phylogenetic trends. Sociobiology. 14:89–156.

Kaib M, Husseneder C, Epplen C, Epplen JT, Brandl R. 1996. Kin-biased foraging in a termite. Proc R Soc Lond B Biol Sci. 263: 1527–1532.

King EG, Spink WT. 1974. Laboratory studies on the biology of the Formosan subterranean termite with primary emphasis on young colony development. Ann Entomol Soc Am. 67:953–958.

Kistner DH. 1985. A new genus and species of *Termitophilous aleocharinae* from mainland China associated with *Coptotermes formosanus* and its zoogeographic significance (Coleoptera: Staphylinidae). Sociobiology. 10:93–104.

Kitade O, Hayashi Y, Kikushi Y, Kawarasaki S. 2004. Distribution and composition of colony founding associations of a subterranean termite *Reticulitermes kanmonensis.* Entomol Sci. 7:1–8.

Lenz M, Runko S. 1993. Long-term impact of orphaning on field colonies of *Coptotermes lacteus* (Froggatt) (Isoptera: Rhinotermitidae). Insectes Soc. 40:439–456.

Matsuura K, Nishida T. 2001. Comparison of colony foundation success between sexual pairs and female asexual units in the termite *Reticulitermes speratus* (Isoptera: Rhinotermitidae). Popul Ecol. 43: 119–124.

Mitton JB. 1993. The correlation between individual heterozygosity of parents and their offspring. Heredity. 71:59–63.

Mitton JB. 1994. Molecular approaches to population biology. Annu Rev Ecol Syst. 25:45–69.

Mitton JB. 1997. Selection in natural populations. New York: Oxford University Press.

Park YI, Bland JM, Raina AK. 2004. Factors affecting post-flight behavior in primary reproductives of the Formosan subterranean termite, *Coptotermes formosanus* (Isoptera: Rhinotermitidae). J Insect Physiol. 50:539–546.

Petrie M. 1994. Improved growth and survival of offspring of peacocks with more elaborate trains. Nature. 371:598–599.

Pillay N. 2002. Father-daughter recognition and inbreeding avoidance in the striped mouse, *Rhabdomys pumilio.* Mamm Biol. 67:212–218.

Pusey A, Wolf M. 1996. Inbreeding avoidance in animals. Trends Ecol Evol. 11:201–206.

Raina AK, Bland JM, Dickens JC, Park YI, Hollister B. 2003. Premating behavior of dealates of the Formosan subterranean termite and evidence for the presence of a contact sex pheromone. J Insect Behav. 16:233–245.

Rantala MJ, Roff DA. 2005. Analysis of the importance of genotypic variation, metabolic rate, morphology, sex and developments time on immune function in the cricket, *Gryllus firmus.* J Evol Biol. 19: 834–843.

Roisin Y. 1999. Philopatric reproduction, a prime mover in the evolution of termite sociality? Insectes Soc. 46:297–305.

Roisin Y, Lenz M. 2002. Origin of male-biased sex allocation in orphaned colonies of the termite, *Coptotermes lacteus.* Behav Ecol Sociobiol. 51:472–479.

Rosengaus RB, Traniello JFA. 1991. Biparental care in incipient colonies of the dampwood termite *Zootermopsis angusticollis* Hagen (Isoptera, Termopsidae). J Insect Behav. 4:633–647.

Rosengaus RB, Traniello JFA. 1993. Disease risk as a cost of outbreeding in the termite *Zootermopsis angusticollis.* Proc Natl Acad Sci USA. 90:6641–6645.

Shellman-Reeve JS. 1994. Limited nutrients in a dampwood termite: nest preference, competition and cooperative nest defense. J Anim Ecol. 63:921–932.

Shellman-Reeve JS. 1999. Courting strategies and conflicts in a monogamous, biparental termite. Proc R Soc Lond B Biol Sci. 266: 137–144.

Shellman-Reeve JS. 2001. Genetic relatedness and partner preference in a monogamous, wood-dwelling termite. Anim Behav. 61:869–876.

Slate J, Kruuk LEB, Marshall TC, Pemberton JM, Clutton-Brock TH. 2000. Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). Proc R Soc Lond B Biol Sci. 267:1657–1662.

Smith DAS. 1981. Heterozygous advantage expressed through sexual selection in a polymorphic African butterfly. Nature. 289:174–175.

Spiess E, Langer B. 1966. Mating control by gene arrangements in *Drosophila pseudoobscura.* Genetics. 54:1139–1149.

Su N-Y, Scheffrahn RH. 1987. Alate production of a field colony of the Formosan subterranean termite (Isoptera: Rhinotermitidae). Sociobiology. 13:167–172.

Thorne BL, Traniello JFA, Adams ES, Bulmer M. 1999. Reproductive dynamics and colony structure of subterranean termites of the genus *Reticulitermes* (Isoptera: Rhinotermitidae): a review of the evidence from behavioral, ecological and genetic studies. Ethol Ecol Evol. 11:149–169.

Tregenza T, Wedell N. 2000. Genetic compatibility, mate choice and patterns of parentage: invited review. Mol Ecol. 9:1013–1027.

Trivers RL. 1972. Parental investment and sexual selection. In: Campell B, editor. Sexual selection and the descent of man. Chicago (IL): Aldine Publishing Co. p. 136–179.

Vargo EL. 2003a. Genetic structure of *Reticulitermes flavipes* and *R. virginicus* (Isoptera: Rhinotermitidae) colonies in an urban habitat and tracking of colonies following treatment with hexaflumuron bait. Environ Entomol. 32:1271–1282.

Vargo EL. 2003b. Hierarchical analysis of colony and population genetic structure in the eastern subterranean termite, *Reticulitermes flavipes*, using two classes of molecular markers. Evolution. 57: 2805–2818.

Vargo EL, Henderson G. 2000. Identification of polymorphic microsatellite loci in the Formosan subterranean termite *Coptotermes formosanus* Shiraki. Mol Ecol. 9:1935–1938.

Vargo EL, Husseneder C, Grace JK. 2003. Colony and population genetic structure of the Formosan subterranean termite, *Coptotermes formosanus*, in Japan. Mol Ecol. 12:2599–2608.

Vargo EL, Husseneder C, Grace JK, Ring D. 2003. Colony and population genetic structure of Formosan subterranean termites from Hawaii and Louisiana. Sociobiology. 41:67–69.

Vargo EL, Husseneder C, Woodson D, Waldvogel MG, Grace JK. 2006. Genetic analysis of colony and population structure of three introduced populations of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the continental United States. Environ Entomol. 35:151–166.

Waldman B. 1988. The ecology of kin recognition. Annu Rev Ecol Syst. 19:543–571.

Watt WB, Carter PA, Donohue K. 1986. Females' choice of 'good genotypes' as mates is promoted by an insect mating system. Science. 233:1187–1189.

Wittenberg JF, Tilson RL. 1980. The evolution of monogamy: hypothesis and evidence. Annu Rev Ecol Syst. 11:197–232.

Woodson WD, Wiltz BA, Lax AR. 2001. Current distribution of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the United States. Sociobiology. 37:661–671.