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Population Biology

Disease risk as a cost of outbreeding in the termite *Zootermopsis angusticollis*

(Isoptera/kinship/inbreeding/social behavior/pathogens)

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ABSTRACT **The effect of the sibship of primary reproductives on mate mortality and the survivorship and growth of incipient colonies was studied in the dampwood termite *Zootermopsis angusticollis*. Males and females paired with nonsibling mates had higher mortality during the first 10–40 days after pairing, although male and female reproductives showed similar patterns of mortality after colony establishment. The source of mortality appeared to be fungal and/or bacterial pathogens. There were no overall differences in the number of eggs and larvae produced by sibling and nonsibling pairs, and no differences in colony size and biomass 4 years after colony establishment. We therefore could not identify any negative effect of inbreeding in the early phases of colony development. Our results suggest that the risk of exposure to pathogens and the ability of termites to locally adapt to disease could influence the genetic identity of primary reproductives and the extent of inbreeding in termite populations.**

Eusociality has evolved in two unrelated insect orders: the Hymenoptera (bees, ants, and wasps) and the Isoptera (termites). Although social organization is highly convergent in these two groups, the mechanisms and preadaptations for eusociality in each order are very different. In the Hymenoptera, one preadaptation for social behavior appears to have been haplodiploidy (1). Termites, on the other hand, are diploid and the same kin selection arguments applied to hymenopteran species cannot explain the advantage for termite workers to forego their own reproduction.

Several hypotheses have attempted to explain the evolution of eusociality in termites (2–5); some current theoretical models focus on the genetic relatedness of colony members and the importance of inbreeding in social evolution. Bartz (3) suggests that termite colonies may have undergone alternating phases of outbreeding and inbreeding. In this model, alate reproductives disperse from the parental colony to mate with genetically unrelated alates, and male and female supplementary reproductives subsequently develop in the parental colony and produce new alates by inbreeding. Inbreeding thus may have facilitated termite social evolution by creating asymmetries in the degrees of relatedness among colony members. There is no consensus, however, on the theoretical effect of inbreeding on the evolution of eusociality, and mathematical models have made conflicting predictions (3, 6–15).

In spite of the potential importance of inbreeding in the evolution of termite social behavior, to our knowledge, no information is available on the consequences of the genetic relatedness of primary reproductives that establish new colonies. Here we report that *Zootermopsis angusticollis* colonies founded by sibling pairs have equivalent fitness to colonies headed by nonsibling primaries but that nonsibling pairs have higher mate mortality during the incipient stages of colony foundation.

METHODS

Termite Collection and Laboratory Colony Establishment. Six stock colonies of the dampwood termite *Z. angusticollis* Hagen were collected during May 1986 at three localities in California: (*i*) the Redwood East Bay Regional Park District, situated 6.8 km from Highway 580, east of Oakland (three colonies nesting in redwood, *Sequoia* sp., and one in bay leaf, *Laurus* sp.; (*ii*) the Palo Alto Foothills Park, 28 km southwest from Highway 101 (one colony nesting in oak, *Quercus* sp.); and (*iii*) the Pebble Beach Resort, located within the Del Monte Forest in the Monterey Peninsula (one colony nesting in pine, *Pinus* sp.). The Redwood population was located approximately 48 km northeast of Palo Alto and 120 km from Pebble Beach; the latter was located 80 km south of the Palo Alto site. Since all stock colonies were collected from small logs (<2.5 m in length), were headed by either primaries (n = 5) or neotenic reproductives (n = 1), and had a single egg pile, termite larvae within a log were considered siblings. Colonies were transferred into plastic boxes and maintained in the laboratory (16). Upon maturation, alates from the six stock colonies were collected and sexed, and their wings were removed. Subsequently, they were randomly paired in plastic Petri dishes (100 × 15 mm) lined with moist paper towel and approximately 6 cm^3 of wood that was continuously replaced as needed. All founding pairs received wood from the same source (*Laurus* sp.) (16). Because we had no information on the genetic relatedness of the termites we collected, we established intra- and interpopulation pairs.

A total of 243 incipient colonies were formed by pairing dealate males and females from either the same colony (sibling pairs, n = 102) or from different colonies (eight combinations of nonsibling pairs from the six stock colonies, n = 141). Because all colonies were not established or censused on the same date, results were standardized by analyzing data based on the time elapsed since pairing.

Mortality and Colony Growth Measurements. Data on the mortality of reproductives and colony growth rates were obtained by examining each incipient colony twice a week for the first 2 months after establishment and approximately once every 10 days for the next 2 months. We then censused the colonies once every 2 months for the next 15 months. Censuses were conducted without knowledge of the colony identity of the primary reproductives. Mortality data for male and female primary reproductives were obtained for 183 incipient colonies; 75 colonies were founded by siblings and 108 colonies by nonsibling mates. Sixty additional colonies were monitored only for colony growth rates. Colony failure occurred when either both reproductives died or the female reproductive died prior to oviposition; all others were con-

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sidered "surviving" colonies (n = 138). Fifteen colonies were removed from this sample for behavioral observations (16) and are not included in the present data set.

Colony growth rates were determined by recording the average number of days elapsed to the appearance of the first egg in the surviving nests as well as the number of eggs and larvae present in the colonies at various time intervals. To determine the long-term effect of sibship, a census was performed on each of the surviving sibling (n = 37) and nonsibling (n = 32) colonies at 4.2 ± 0.4 years (range, 3.6–4.6 years) after the original primary reproductives were paired. Colony biomass was determined only during the fourth-year census. The combined offspring wet weight (all larvae, soldiers, and eggs) was measured with a Mettler AE163 balance. The biomass of reproductives was obtained by weighing both primary reproductives or supplementary reproductives present.

Statistical evaluations of the differences in mortality among mates were made by using the SAS Lifetest procedure (17).

RESULTS

Mortality Rates. The highest mortality of primary reproductives occurred soon after establishment, reaching approximately 43% at 100 days after pairing (Fig. 1). Neither males nor females differed significantly in their mortality trends (P > 0.05, ref. 17). Fungal and/or bacterial infections seemed to be important sources of mortality as shown by the time course of mortality, the presence of fungal growth on the bodies of dead individuals, and the appearance of the exoskeleton (18). An analysis of the time course of mortality showed that females in nonsibling pairs had significantly higher mortality at 10, 20, and 40 days after pairing (z = 2.4, 2.1, and 1.71, respectively; P < 0.05; Fig. 1A). Similarly, males suffered significantly higher mortality in nonsibling pairs than in sibling pairs during the first 10 and 20 days after pairing (z = 2.7 and 1.9, respectively; P < 0.05; Fig. 1B). To test whether these differences in mortality were primarily due to reproductives originating from diseased colonies, a covariate analysis was performed. No association between colony origin of the primary reproductives and male or female mortality was found (χ^2 = 1.9; P > 0.05). Collection site, sibship, and the intra- and interpopulation nature of the pairings also had no effect on mate mortality (χ^2 = 3.2, 3.6, and 3.6, respectively; P > 0.05; ref. 17). For example, within the Oakland site alone the mortality of males and females in sibling pairs was significantly less than in nonsibling pairs (z = 2.17 and 1.75, respectively; P < 0.05). The fact that significant association among these variables and mortality was lacking and that most of the stock colonies survived and produced alates in the laboratory for at least 2 years suggests that reproductives originated from healthy stock colonies.

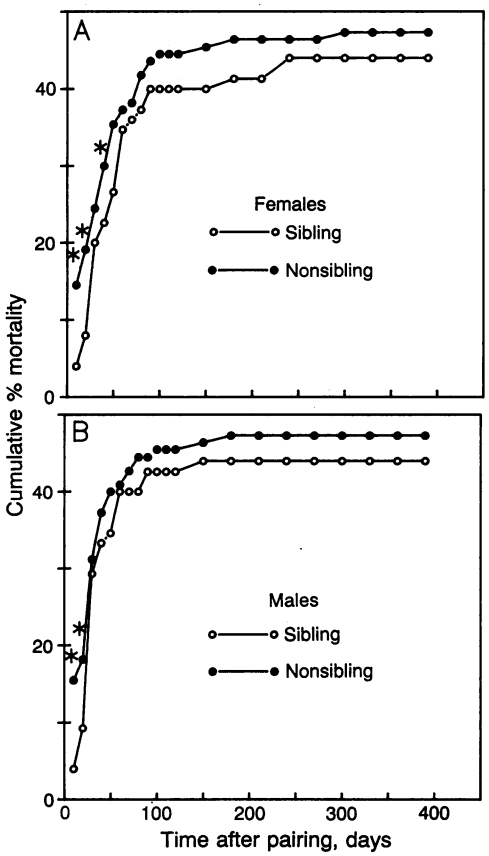

FIG. 1. Cumulative percent mortality for females (A) and males (B) paired with either sibling or nonsibling mates during the incipient stages of colony foundation. Asterisks denote a significant difference between points at P < 0.05.

Approximately 4 years after colony establishment, mortality in nonsibling-founded colonies was significantly higher than in colonies founded by sibling pairs (G = 4.8; df = 1; P < 0.05; ref. 19 and Table 1). The consequence of male or female mortality on the colony's reproductive output and mate survival 4 years after establishment was also analyzed for sibling and nonsibling pairs. At the time of the death of the female primary, sibling colonies had produced significantly more offspring (eggs and larvae) than nonsibling pairs, and mate survival after the female's death was longer for males in sibling than in nonsibling pairs (Table 2). On the other hand, the consequence of male mortality prior to female mortality did not differ between sibling and nonsibling pairs.

Egg Production. Of the total 243 incipient colonies, 123 (50.6%) produced at least one egg during the first 6 months after pairing. Fifty-three percent of the original sibling pairs and 48% of the nonsibling pairs produced at least one egg during this period, but this difference was not statistically significant (P > 0.05, G test). There was considerable variation in the time at which females began oviposition, as shown in the average time (±SD) to the appearance of the first egg (47 ± 36.2 days; range, 15–271 days) for both sibling and nonsibling colonies. The average number of eggs (±SD) first observed during the census period was 2.6 ± 1.8 (range, 1–9 eggs). Neither the average time to initiate egg laying nor the number of eggs first seen during the census period were significantly different for sibling and nonsibling pairs (P > 0.05, Mann–Whitney U test). The average egg production for

Table 1. Mortality and colony dynamics in sibling- and nonsibling-founded colonies during the fourth-year census

Parameter	Sibling-founded colonies	Non-sibling-founded colonies	P
% mortality	51.3	71.2	* (G = 4.8)
Eggs, no.	6.9 ± 2.5 (n = 37)	4.1 ± 2.0 (n = 32)	NS
Larvae, no.	12.6 ± 2.5 (n = 37)	17.1 ± 2.3 (n = 32)	NS
Brood weight, g	0.3 ± 0.04 (n = 37)	0.3 ± 0.05 (n = 32)	NS
Reproductive weight, g	0.1 ± 0.01 (n = 37)	0.1 ± 0.03 (n = 32)	NS

Asterisk denotes a significant difference between points at P < 0.05. Analysis for differences in percent mortality was performed by a G test; differences among averages were performed by a Wilcoxon sum-rank test (17). n, 75 for sibling-founded colonies; 108 for non-sibling-founded colonies. Data are average ± SE.

Table 2. Reproductive output and mate survival in sibling- and non-sibling-founded colonies after the death of the primary reproductive male or female

Parameter	Sibling/nonsibling	
	Males dying prior to females	Females dying prior to males
Eggs, no.	5.0 ± 2.3/1.8 ± 1.0^{NS}	4.1 ± 2.2/0.1 ± 0.1**
Larvae, no.	9.2 ± 6.4/5.1 ± 2.3^{NS}	16.0 ± 7.0/2.7 ± 2.4*
Days mate survived since establishment	186.0 ± 65.9/168.0 ± 47.6^{NS}	354.5 ± 127.0/161.7 ± 57.4*

Data are average ± SE. Statistical analysis for differences in averages were performed with a Wilcoxon sum rank test. *, $P < 0.1$; **, $P < 0.05$; NS, no statistical significance.

39 sibling and 57 nonsibling pairs during the first year of colony establishment showed no overall significant differences ($P > 0.05$, Mann–Whitney U test; Fig. 2). At 180 days after pairing, however, sibling pairs had laid significantly more eggs than nonsibling pairs ($P = 0.006$, Mann–Whitney U test), and a trend toward significant differences in egg production was also observed at 300 days ($P = 0.06$, Fig. 2). That most of the mortality of primary reproductives occurred prior to 180 days (Fig. 1) and the average number of offspring first observed in colonies where the queen died prior to 180 days was not significantly different from the average number of offspring first observed in those colonies where the female reproductive survived up to 180 days ($\bar{x} = 2.5 \pm 1.4$ vs. 2.5 ± 1.8 offspring; $P > 0.05$, Wilcoxon sum rank test) suggests that the differences in egg production at this time were not due to the higher mortality of nonsibling pairs.

The significant differences in egg production at 180 days after pairing did not continue through time. During the fourth year, the average number of eggs present in sibling and nonsibling colonies was not significantly different (Table 1).

Production of Larvae. Differences in egg production between sibling and nonsibling pairs did not translate into differences in patterns of colony growth. The average number of larvae was not consistently higher in sibling-pair colonies and there were no overall significant differences in the production of larvae ($P > 0.05$, Mann–Whitney U test; Fig. 3). Six months after pairing the average (±SD) brood production (egg and larvae) was 23.0 ± 10.6 individuals. During the fourth year, there was no difference in the average number of larvae produced by sibling and nonsibling pairs (Table 1).

Colony Biomass. Colonies originally headed by sibling and nonsibling pairs showed no significant differences in brood mass (larvae and eggs) 4 years after establishment (Table 1). The average reproductive mass (either the original primary pair or supplementary reproductives) was also not significantly different between colonies founded by sibling and nonsibling pairs.

Production of Supplementary Reproductives. After 4 years, 66% of the sibling-founded colonies had one or both primary reproductives replaced by supplementary neotenic reproductives (20), and 54.5% of the originally nonsibling headed colonies had supplementaries. These differences were not statistically significant ($G = 0.8$; df = 1; $P > 0.05$). Hence, the presence of supplementary reproductives was independent of mate identity.

DISCUSSION

The higher mortality of males and females in nonsibling pairs during the first 40 days of colony foundation and 4 years after colony establishment suggests that outbreeding in *Z. angusticollis* entails considerable costs. The pattern of mortality we have described is not due to nutrition (ref. 21 and unpublished data).

It is likely that mate mortality in *Z. angusticollis* involves disease. Fungi and bacteria have been described as important agents of termite disease (18) and are capable of producing high mortality within the first 10–15 days of contact (18, 22–32), a pattern very similar to our present results. Fungi may produce termite toxins (27) and feeding inhibitors (26) or they may be entomophagous. In many incipient colonies showing signs of decline, fungi were observed on wood, fecal pellets, and the bodies of dead termites, but bacteria or bacterial toxins may also have been pathogenic.

Additional data support the hypothesis that pathogens caused the observed mortality. We established 23 primary reproductive pairs using one alate from a known healthy stock colony and one known to be infected. In all 23 replicates, there was 100% mortality of the previously healthy nonsibling mate within the first 10 days after pairing, and there were obvious signs of infection on the bodies of termites. These experimental results suggest that pathogen transfer between primary reproductives is indeed possible and that it may explain our mate mortality data.

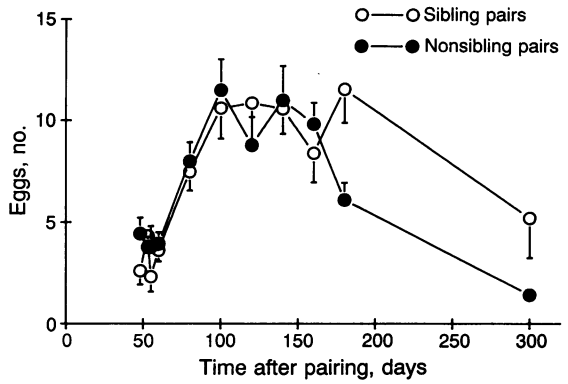

FIG. 2. Egg production rate in incipient colonies founded by sibling and nonsibling mates. Data are the average ± standard error. Data points with no error bar have a standard error too small to be graphed.

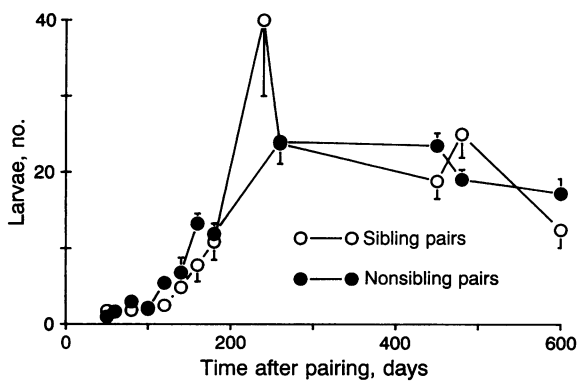

FIG. 3. Production of larvae in incipient colonies founded by sibling and nonsibling mates. Data are the average ± standard error. Data points with no error bar have a standard error equal to zero or too small to be graphed.

The observed pattern of differential mate mortality between sibling and nonsibling primary reproductives may be due to genetically based resistance or acquired immunity to pathogens. Although we have not examined variability in the response of the offspring of inbred and outbred pairs to pathogens, the hypothesis of acquired immunity appears to be supported by our results. Sibling primary reproductives may be immune to the same pathogens due to prior exposure while maturing in the same colony. When sibling alates are paired, they show low mortality because they have specific immunological memory for the "familiar" pathogens associated with a mate. Nonsiblings, in contrast, may have acquired immunity to different pathogens present in different colonies. Immune individuals thus transmit pathogens to not-yet immune individuals, leading to mortality. Such differences in acquired immune response would explain the higher mortality of nonsibling pairs but the lack of differences in the production of larvae in the surviving colonies. That is, surviving pairs of primary reproductives that can resist pathogens through acquired immunity have equal colony growth rates, whether or not they are inbred or outbred.

Some insects, including roaches, exhibit complex immunological capabilities, generating antibodies toward bacteria and toxins (33–35). Termites are hemimetabolous and relatively long-lived and it is reasonable to expect some similarities between their immunological response and that of the phylogenetically related roaches (33). Nestmates might be "socially immunized" against pathogens by receiving innoculae in proctodeal and stomodeal exchanges and through allogrooming, thus facilitating an acquired immune response at the colony level.

In addition to ecological constraints, such as predation on alates (36–39), the use of wood as a food source (40–43), and climatic factors (44), disease risk may have restricted the extent of outbreeding in termite populations and favored the relatively local dispersal of primary reproductives (9) and/or the serial inbreeding of supplementary reproductives. Termites may have thus locally adaptated to pathogens via genetically based or acquired immunity, resulting in specific adaptive host–pathogen associations that could be compromised by outbreeding (45–49). The fact that sibship-related mate mortality occurs within a *Z. angusticollis* population suggests that the causal agents of mortality vary on a spatial scale comparable to the actual dispersal capabilities of alates (50–60).

It has been suggested that genetic asymmetries might have influenced the evolution of sterility in the soldier and worker castes of higher termites (3, 4, 60, 61), although genetic biases do not appear to mediate social interactions in extant species (50, 62–65). In addition to such intrinsic factors, termites are predisposed to eusociality due to their monogamous mating system (42), claustral colony foundation (66), and hemimetabolous development that permits immature insects to provide colony labor (60). In the models of Bartz (3) and Hamilton (40), the underbark or rotting wood habitat of ancestral termites is considered to be confining and thus enforces inbreeding. Based on our results in *Z. angusticollis*, we propose that disease risk may also have promoted inbreeding, and susceptibility to pathogens may explain why inbreeding appears to be relatively common in termites. Bartz's theory of the origin of termite eusociality (3) suggests that outbreeding must occur at the colony level to generate genetic asymmetries favorable to social evolution. Alternating cycles of inbreeding and outbreeding may still occur under the constraint of exposure to disease. Pathogens appear to decrease the success of outbred pairs and set additional limits on dispersal.

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1. Hamilton, W. D. (1964) *J. Theor. Biol.* **7**, 1–52.
2. Cleveland, L. R., Hall, S. R., Sanders, E. P. & Collier, J. (1934) *Mem. Am. Acad. Arts Sci.* **17**, 185–342.
3. Bartz, S. H. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5764–5768.
4. Lacy, R. C. (1980) *Am. Nat.* **116**, 449–451.
5. Leinass, H. P. (1983) *Am. Nat.* **121**, 302–304.
6. Wade, M. J. (1980) *Evolution* **34**, 844–855.
7. Wade, M. J. & Breden, F. (1981) *Evolution* **35**, 844–858.
8. Shields, W. M. (1982) *Evol. Theory* **5**, 245–279.
9. Shields, W. M. (1984) in *The Ecology of Animal Movement*, eds. Swingland, I. R. & Greenwood, P. J. (Calderon, Oxford), pp. 132–159.
10. Shields, W. M. (1982) *Philopatry, Inbreeding and the Evolution of Sex* (State Univ. of New York Press, Albany, NY), p. 233.
11. Michod, R. (1979) *J. Theor. Biol.* **81**, 223–233.
12. Michod, R. (1980) *Genetics* **96**, 275–296.
13. Craig, R. (1982) *J. Theor. Biol.* **94**, 119–128.
14. Pamilo, P. (1984) *Behav. Ecol. Sociobiol.* **15**, 241–248.
15. Uyenoyama, M. K. (1984) *Evolution* **38**, 778–795.
16. Rosengaus, R. B. & Traniello, J. F. A. (1991) *J. Ins. Behav.* **4**, 633–647.
17. SAS Institute (1985) *Statistics Version 5* (SAS Institute, Cary, NC), p. 956.
18. Sands, W. L. (1969) in *Biology of Termites*, eds. Krishna, K. & Weesner, F. (Academic, New York), Vol. 1, pp. 495–524.
19. Sokal, R. R. & Rohlf, J. F. (1981) *Biometry* (Freeman, New York), p. 859.
20. Castle, G. B. (1934) in *Termites and Termite Control*, ed. Kofoid, C. A. (Univ. of California Press, Berkeley, CA), pp. 273–310.
21. Shellman-Reeve, J. S. (1990) *Behav. Ecol. Sociobiol.* **71**, 389–397.
22. Amburgey, T. L. (1979) *Sociobiology* **4**, 279–296.
23. Gilbertson, R. L. (1984) in *Fungus-Insect Relationships: Perspectives in Ecology and Evolution*, eds. Wheeler, Q. & Blackwell, M. (Columbia Univ. Press, New York), pp. 130–165.
24. Williams, R. M. C. (1959) *Insectes Soc.* **6**, 291–304.
25. Vypiyach, A. N. & Voronika, G. U. (1972) in *Termites*, ed. Zolotarev, E. K. (Moscow Univ. Publishing House, Moscow), pp. 193–201.
26. Amburgey, T. L. & Beal, R. H. (1977) *Sociobiology* **3**, 35–38.
27. Kovoor, J. (1964) *Bull. Biol. Fr. Belg.* **98**, 491–510.
28. Toumanoff, C. (1966) *Insectes Soc.* **13**, 155–164.
29. Alston, R. A. (1947) *Nature (London)* **160**, 120.
30. Lund, A. E. & Engelhardt, N. T. (1962) *J. Insect Pathol.* **4**, 131–132.
31. Toumanoff, C. (1966) *Insectes Soc.* **13**, 155–164.
32. Kramm, K. R., West, D. F. & Rockenbach, P. G. (1982) *J. Invert. Pathol.* **40**, 1–6.
33. Dunn, P. E. (1990) *BioScience* **40**, 738–744.
34. Dunn, P. E. (1991) in *Phylogenesis of Immune Functions*, eds. Warr, G. W. & Cohen, N. (CRC, Boca Raton, FL), pp. 19–44.
35. Karp, R. D. & Duwel-Eby, L. E. (1991) in *Phylogenesis of Immune Functions*, eds. Warr, G. W. & Cohen, N. (CRC, Boca Raton, FL), pp. 1–18.
36. Basallingappa, S. (1970) *Indian Zool.* **1**, 45–50.
37. Sheppe, W. (1970) *Insectes Soc.* **17**, 205–218.
38. Deligne, J., Quennedy, A. & Blum, M. S. (1981) in *Social Insects*, ed. Hermann, H. R. (Academic, New York), Vol. 2, pp. 2–76.
39. Dial, K. P. & Vaughan, T. A. (1987) *Biotropica* **19**, 185–187.
40. Hamilton, W. D. (1978) in *Diversity of Insect Faunas*, eds. Mound, L. A. & Waloff, N. (Halsted, New York), pp. 154–175.
41. Nalepa, C. A. & Jones, S. C. (1991) *Biol. Rev.* **66**, 83–97.
42. Nalepa, C. A. (1984) *Behav. Ecol. Sociobiol.* **66**, 83–97.
43. Nalepa, C. A. (1984) *Behav. Ecol. Sociobiol.* **14**, 273–279.

44. Clement, J. (1981) in *Biosystematics of Social Insects* (Academic, New York), pp. 49–61.
45. Edmunds, G. F. & Alstad, D. N. (1978) *Science* **199**, 941–945.
46. Templeton, A. R. (1986) in *Conservation Biology*, ed. Soule, M. E. (Sinauer, Sunderland, MA), pp. 105–116.
47. Alstad, D. N. & Edmunds, G. F. (1983) *Science* **220**, 93–95.
48. Alstad, D. N. & Edmunds, G. F. (1987) *Ann. Entomol. Soc. Am.* **80**, 692–701.
49. Hoffman, A. A., Turelli, M. & Simmons, G. M. (1986) *Evolution* **40**, 692–701.
50. Luykx, P. (1985) in *Caste Differentiation in Social Insects*, eds. Watson, J. A. L., Okot-Kotber, B. M. & Noirot, C. (Pergamon, New York), pp. 17–25.
51. Minnick, D. R. (1973) *Environ. Entomol.* **2**, 587–591.
52. Grasse, P. P. & Noirot, C. (1955) *Insectes Soc.* **16**, 213–220.
53. Herfs, A. (1952) *Z. Angew. Entomol.* **33**, 69–77.
54. Weesner, F. M. (1958) *Ann. Rev. Entomol.* **5**, 153–170.
55. Harris, W. V. & Sands, W. A. (1964) *Symp. Zool. Soc. London* **14**, 113–131.
56. Jones, S. C., La Fage, J. P. & Wright, V. L. (1981) *Sociobiology* **6**, 221–242.
57. Grassi, B. & Sandias, A. (1896) *Q. J. Microsc. Sci.* **39**, 245–322.
58. Grassi, B. & Sandias, A. (1896) *Q. J. Microsc. Sci.* **40**, 1–82.
59. Reilly, L. M. (1987) *Am. Nat.* **130**, 339–349.
60. Myles, T. G. & Nutting, W. L. (1988) *Q. J. Microsc. Sci.* **63**, 1–23.
61. Syren, R. M. & Luykx, P. (1977) *Nature (London)* **266**, 167–168.
62. Luykx, P. (1986) *Insectes Soc.* **33**, 221–248.
63. Luykx, P. & Luykx, J. M. (1986) *Insectes Soc.* **33**, 406–421.
64. Crozier, R. H. & Luykx, J. M. (1985) *Am. Nat.* **126**, 867–869.
65. Hahn, P. D. & Stuart, A. M. (1987) *Sociobiology* **13**, 83–92.
66. Nutting, W. L. (1969) in *Biology of Termites*, eds. Krishna, K. & Weesner, F. (Academic, New York), Vol. 1, pp. 49–88.