Chapter 21

Molecular Genetic Methods: New Approaches to Termite Biology

C. Husseneder[1], E. L. Vargo[2], and J. K. Grace[1]

[1]Plant and Environmental Protection Sciences, University of Hawaii at Manoa, 3050 Maile Way, Gilmore 310, Honolulu, HI 96822
[2]Department of Entomology, North Carolina State University, Campus Box 7613, Raleigh, NC 27695

Termites (Isoptera) are of global economic and ecological importance as decomposers of lignocellulose matter. Yet little is known about their biology due to their cryptic feeding and nesting habits. The advent of a variety of molecular genetic techniques provides a powerful method to elucidate many aspects of termite ecology and social organization. We present an overview of currently applied molecular genetic methods, including analyses of proteins, mitochondrial and genomic DNA, in a variety of termite species. These methods complement each other in the description of termite population structure, the identification of colonies and their breeding systems. The increasing application of these and other methods will yield a much improved understanding of termite ecology and social evolution as well as more effective means of controlling pest species.

Husseneder, C., E. L. Vargo, and J. K. Grace. 2003. Molecular genetic methods: new approaches to termite biology. *In:* Wood Deterioration and Preservation: Advances in Our Changing World (B. Goodell, D. D. Nicholas, and T. P. Schultz, editors). American Chemical Society Symposium Series 845: 358-370.

A Short Introduction to Termite Biology

Termites are of immense ecological and economic importance. Ecologically, termites are the primary insect group adapted to consume wood and dry plant matter (lignocellulose), and to turn this nutritive-poor matter into protein biomass. With similar efficiency termites consume urban wooden structures and agricultural crops. Although only about 83 among the 2700 termite species are pests, they severely impact tropical and subtropical regions. In the USA subterranean termites of the genus *Reticulitermes* spp. and *Coptotermes* spp. are the most destructive. Subterranean termites are established in every region of the contiguous United States and Hawaii, costing an estimated $2 billion per year for damage repair and management efforts (*1*). There is growing concern about the economic impact of termites on urban structures and agricultural crops, calling for a combination of preventative and remedial measures to control colonies, as well as regulatory actions to reduce the chance of new introductions and limit the further spread of termite populations. Improvement on both levels requires a thorough understanding of termite biology.

The main features of termite biology, such as their colonial life-style, cooperative tasksharing and the monopolization of reproduction by a limited number of breeders (kings and queens), are linked to their evolution as social insects. Compared to research on other social insects (ants, bees and wasps), termite sociality has been somewhat neglected. Recent studies, however, have proven that termites provide valuable alternative and complementary models to the understanding of social evolution (*2*). Description of termite sociality requires detailed knowledge of the genetic variation within and between colonies, i. e. the population's genetic structure. These genetic patterns depend on, as well as reflect, the dispersal mode, distinctness of colonies and the breeding system (*3*).

A frequent problem in termite research is the cryptic life-style of termites. Because most termites live entirely hidden underground or inside wooden timbers or trees, direct observation under natural conditions is at best limited. Sometimes the only indication of their presence are swarming events, when a considerable number of winged reproductives are released from their mother colony to found new colonies. Besides swarming, termite colonies can proliferate by "budding", i.e., mature colonies containing multiple kings and queens form satellite nests that may eventually become independent colonies. This creates complicated colony structures consisting of widespread interconnected foraging areas and multiple nests containing a variable number of reproductives. Moreover, different levels of inbreeding in colonies, depending on the degree of genetic relatedness of reproductives, in turn affects

the genetic make-up of the population (*3*). Due to the termites' hidden nesting and foraging habits these fundamental patterns and processes in termite biology are still literally hidden in the dark (*2, 4*). However, important features of insect biology, such as population structure, dispersal patterns, colony identity and social organization, can be inferred from the genetic structure of colonies and populations (*3, 5*).

Molecular Genetic Research in Termites

Recently, molecular genetic methods have been employed to gain insight into termite phylogeny (e.g., *6, 7*) and population genetic patterns (*4, 8-20*). In this chapter we review the molecular genetic approaches to population and colony genetic structure of termites. We first explain the basic features of the molecular methods used in termite research. We then address what information these methods provide to answer questions concerning termite population genetics and colony organization, drawing on examples from a variety of termite species. We will emphasize subterranean termites, especially *Reticulitermes* spp. and the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), because these are the most severe and broadly distributed pest species in the US and worldwide and are therefore primary targets for improved research and management.

The Methods

In the last decade, a rapidly increasing palette of molecular techniques has been developed to address a wide range of questions (*21, 22*). Which technique is most appropriate depends on the amount of genetic variability and "resolution power" needed to answer a particular question. Molecular markers currently used in termite research are (1) alloenzymes, (2) mitochondrial DNA and (3) genomic DNA, all of which have different characteristics and inheritance patterns and therefore target different but overlapping research areas.

(1) Diploid organisms, such as termites, have two copies of each genetic region (locus) on homologous pairs of chromosomes, called alleles. Mutations in genes coding for enzymes create a variety of different alleles in a population. The resulting gene products (proteins) have the same function but different structure and are called alloenzymes. Due to their structural differences and enzymatic activity, alloenzymes can be separated through electrophoresis and detected by enzyme specific stains (*23*). Alleles are inherited according to Mendel's rules and are presumed to be selectively neutral. Therefore the particular pattern of allelic distribution within and among colonies reflects relatedness and inbreeding of populations and colonies as well as the colonies' social structure, i.e., numbers and relatedness of reproductives (e.g., *9, 15, 16, 24*).

(2) Mitochondrial DNA is a double-stranded ring of extrachromosomal DNA located in the mitochondria, the organelles of cell respiration. Mitochondrial DNA reaches about 16-20kb in size (*25*). It lacks efficient DNA repair mechanisms, so populations contain a variety of different types of mitochondrial DNA, called haplotypes. Differences between haplotypes can be detected through length heterogeneity, restriction fragment analysis, double strand conformation polymorphism or sequencing of mitochondrial DNA regions (*26*). Mitochondrial DNA is inherited in a non-Mendelian, cytoplasmic fashion, generally through maternal transmission only. This marker type is therefore used for identifying the distribution of matrilines within populations and colonies (e.g., *14, 16, 19, 24*).

(3) Genomic DNA provides powerful markers for fine-grained analysis of genetic variation. Termite genomic DNA is currently analyzed by two methods, multilocus DNA fingerprinting and microsatellite genotyping (*13, 18, 20, 27-31*). Multilocus DNA fingerprinting and microsatellite genotyping have excellent resolution power because they target simple repetitive sequences with high mutation rates in non-coding regions of the genome. DNA fingerprinting detects barcodelike banding patterns by restriction of genomic DNA and hybridization with labeled probes visualized by autoradiography or chemiluminescence. Microsatellite genotyping uses polymerase chain reaction to amplify alleles containing repetitive sequences. Alleles vary in length according to the variable number of repeats and are co-dominant. Thereby the proportion of heterozygotes (individuals carrying different alleles at gene loci on corresponding chromosomes) in colonies and populations can be detected and analyzed using the standard statistical procedures of population genetics (e.g., *F*-Statistics). DNA fingerprints, as well as microsatellite genotypes, are inherited according to Mendelian rules and therefore contain information about ancestries and relationships of individual termites, colonies and populations (methods reviewed by 22, *26*).

Population Differentiation and Dispersal of Termites

With increasing globalization due to trade, commerce, and tourism, alien species rapidly spread worldwide. The introduction of exotic species to an area can pose threats to the local ecology, e.g., through displacement of native species, as well as cause significant economic problems. Exotic termites, such as the Formosan subterranean termite, have invaded over a dozen states of the

USA, inflicting costs of at least $1 billion/year for damage repair and control. Regulatory efforts, such as inspections and quarantines, need to be targeted at the most common routes of introduction. Therefore, it is essential to understand the sources and routes of termite introduction, as well as the means by which subsequent spread through a habitat takes place. To date, there is much speculation but little known about the numbers and sources of introduction and ways of spread of termites. The key to understanding how termite colonies establish and proliferate lies in molecular genetic research on termite populations, including native as well as introduced species. Yet, in contrast to other insect pests, only a few such studies have been conducted on termites (*3, 5*).

The first genetic marker used to investigate genetic differentiation of termite populations were alloenzymes. Depending on the species and geographic range, the percentage of polymorphic alloenzyme loci and, therefore, their resolution power and applicability to particular questions differ [*Nasutitermes nigriceps* 8% (*15*); *C. formosanus* 12%-17% (*10, 11*); *Incisitermes schwarzi* 17% (*32*); *Reticulitermes flavipes* 29% (*9*); *Reticulitermes* spp. 52% (*33*); *Hodotermopsis* spp. 49% (*34*); *Zootermopsis* spp. 63% (*35*)].

In most cases the degree of polymorphism has been sufficient to describe the geographic differentiation between species or subspecies, e.g., of *Reticulitermes* spp. in Europe (*33*), *Hodotermopsis* spp. of Japan and China (*34*) or populations of *Coptotermes acinaciformis* with different nesting behaviors (mound-building versus subterranean) in Australia (*36*).

Beyond geographic distribution, description of genetic differentiation within and among populations is important for investigating the patterns and processes of how termites disperse through an area. Alloenzyme analysis in a native subterranean termite, *R. flavipes,* detected no significant genetic differentiation among sites in central Tennessee. However, alloenzyme frequencies differ between colonies (*9, 37*). Therefore the lack of increasing genetic differentiation over geographical distance was not attributed to a overall lack of genetic diversity but to considerable dispersal potential of winged reproductives resulting in homogenization of the population genetic structure. High dispersal capability might also explain the low mitochondrial DNA divergence among sites spanning across Georgia (*19*).

Alloenzyme studies of *C. formosanus*, an introduced invasive termite pest in the USA, revealed moderate genetic differentiation between geographically separated populations, such as Hawaii, Florida and Louisiana (*10*). In a subsequent study, Wang and Grace (*38, 39*) included southern China, the presumed origin of *C. formosanus,* in their analysis. Based on the genetic distances between the populations, multiple introductions on the US mainland were suggested with Hawaii and Lake Charles (Louisiana) being genetically similar to the native Chinese populations while New Orleans (Louisiana) and

Florida seem to have remained genetically isolated from the other group. However, the small sample size of colonies per population and populations per region in this study makes it difficult to pinpoint the actual source of introduction or ways of spread.

On a smaller geographic scale, such as the Hawaiian islands, no genetic differentiation was detected between colonies and populations of *C. formosanus.* All 8 investigated alloenzyme loci were monomorphic (*11*). A similar lack of genetic differentiation between Hawaiian populations was reported by Broughton and Grace (*12*) based on restriction analysis of mitochondrial DNA. Because *C. formosanus* is an introduced species, this lack of genetic variation in Hawaii might be due to a single introduction, multiple introductions from related sources, or bottlenecks (e.g., through successful termite control efforts). However, because of the limited polymorphism of alloenzymes and of mitochondrial DNA restriction sites, conclusions based on these methods are tentative. The application of methods with higher resolution power, such as genomic DNA markers is needed for a more detailed understanding of the genetic relationships within and among populations.

In contrast to alloenzymes and mitochondrial DNA, genomic DNA markers (DNA fingerprints and microsatellite genotypes) have revealed considerable genetic polymorphism even in introduced termite species on small spatial scales, such as *C. formosanus* on the island of Oahu, Hawaii. We have recently found that colonies were clearly genetically differentiated, yet the genetic distance between them showed no correlation with geographic distance, as in *Reticulitermes* (see above). Given the presumed weak flight capacity of *C. formosanus,* hindered further by mountain ranges dividing the island of Oahu, this lack of isolation by distance is surprising. It suggests that the predominant method of spread even on a fairly small scale is not by natural means but is human mediated (Husseneder, Vargo, Grace unpubl.). In contrast to the findings in *Reticulitermes* spp. and *C. formosanus*, natural populations of a termite species from the same family, the African subterranean termite *Schedorhinotermes lamanianus*, do show isolation by distance in spatial scales below their swarming distance of about 1 km apparently due to limited alate dispersal (*18*).

To trace ways and speed of dispersal and to track possible source populations of *C. formosanus*, we are currently assembling a large data base, measuring genetic interrelationships within and among introduced and native populations from the USA, Japan and China. First data show a lower genetic diversity in introduced populations, probably due to genetic bottlenecks reflecting the introduction event and ongoing control efforts. Nevertheless, genetic differentiation between populations is sufficient to classify individuals to source populations (Husseneder, Vargo, Grace unpubl.).

Population genetics in termites is still in its early days and only a few aspects in a few species have been studied so far. These studies indicate a wide range in intraspecific genetic variability, degrees of population differentiation and dispersal modes. A more complete picture will require detailed comparative studies involving natural and introduced populations of a variety of termite species.

Genetic Identification of Termite Colonies

To interpret genetic differentiation at the level of the population it is important to understand how a termite colony is organized, because colony identity and breeding systems shape the local population structure. Moreover, from a termite management point of view, the colony is the target unit. Therefore, it must be clearly identified and its structure described. Yet, because mark-release-recapture of dyed termites to connect termites from different collection sites to foraging areas of colonies is time consuming and requires large numbers of termites at each site, the affiliation of collection (or infestation) sites to particular colonies prior to control efforts is rarely known. In addition, if termites re-appear after control in the same location it is not known from which colony they originate. Molecular methods make it possible to assign termite collection sites to colonies and to determine the origin of re-appearing termites.

In termite species that exhibit sufficient genetic variation within populations colonies can be segregated by alloenzyme and mitochondrial DNA analysis. For example, alloenzyme studies found that colonies of *R. flavipes* are genetically isolated from adjacent colonies (*4, 9*). Using genetic differentiation between colonies, Bulmer et al. (*24*) were able to determine colony affiliation of sampled termites using alloenzymes and mitochondrial DNA haplotypes.

However, in other termite species alloenzymes and mitochondrial DNA markers failed to reveal genetic variation between colonies. For example, restriction fragment analysis of mitochondrial DNA, showed no genetic differentiation between subspecies of *Zootermopsis nevadensis* nor between colonies within a proposed subspecies (*40*). Especially in introduced species, such as *C. formosanus,* alloenzyme and mitochondrial DNA markers are not polymorphic enough to reveal colony differentiation on a small scale. In Hawaiian *C. formosanus* populations no restriction fragment variation of mitochondrial DNA and only one polymorphic alloenzyme locus was discovered (*11, 12, 34*). Therefore more variable DNA regions, such as repetitive sequences of genomic DNA, had to be employed to detect genetic differentiation between colonies.

Husseneder et al. (*18*) used multilocus DNA fingerprinting to differentiate colonies of the African subterranean termite *S. lamanianus* even on a small geographic scale. We employed this same method to describe colonies of *C. formosanus*. In *C. formosanus* populations from Hawaii and Louisiana genetic similarities within colonies were significantly higher than genetic similarities between termites from different colonies. Colonies can thus be genetically differentiated. Due to this differentiation, termites can be assigned to colonies by looking for the closest match with the highest genetic similarity reflected by the proportion of shared bands. In terms of colony assignment molecular methods proved to be superior to behavioral tests and morphometric studies in *C. formosanus* (*41-43*). In addition, the genetic profile accumulated from pools of colony members is specific for a colony. Provided there is sufficient genetic differentiation between colonies of a local population, diagnostic bands distinguish the genetic profile of the target colony from the profiles of other colonies. Termites that possess these bands can be assigned to a particular colony. In addition to assigning termites to colonies, genetic profiles can be used to "tag" colonies prior to elimination for re-infestation studies. In cases where termites appear in the same location after elimination through baiting, it can be decided - by comparing genetic profiles - if they are remnants from the same colony, invaders from neighboring colonies or – if the profiles do not match known colonies - new infestations from outside the area. Genetic profiles combining multiple microsatellite loci can be used for the same purpose (Vargo and Husseneder unpubl.).

In sum, depending on the genetic variation in a termite population, alloenzymes, mitochondrial DNA and/or genomic DNA markers can be used to group termites from different collection sites to colonies and thus give an estimation of the foraging area of a colony. Moreover, it is possible to monitor the success of colony elimination efforts by showing if activity at all collection sites belonging to a colony ceases and verifying the origin of re-appearing termites. Lastly, defining a colony is a necessary first step for investigating breeding systems and intracolonial structures.

Social Organization and Intracolonial Structures

Surprisingly little is known about social and spatial organization of termite colonies. Termite colonies are not always simple families consisting of one breeding pair. Polygamy can arise either from multiple adults co-founding a colony (pleometrosis) or from multiple reproductives reared within a colony. The origin and relatedness of breeders determines the degree of inbreeding within a colony. Molecular methods make it possible to assess whether a single pair or multiple kings and queens are reproducing within a colony, the degrees

of inbreeding between them and whether the kin composition leads to genetic structure within the colony.

Mitochondrial DNA is passed on through matrilines only. Therefore, it can be used to detect the number of different matrilines in colonies and thus the number of maternally unrelated females. Mitochondrial DNA haplotypes were investigated to determine the origin of co-occurring kings and queens in colonies of several termite species. Distinct haplotypes can occur due to several reasons. Heteroplasmy (different mitochondrial haplotypes within the same individual) and paternal contribution of mitochondrial DNA are considered rare (*40*). Queen adoption or mixing of different colonies resulting in multiple haplotypes at the same collection site are discussed (*44*), however not yet unambiguously proven for termites. Thus, colony foundation by multiple maternally unrelated queens remains the most likely hypothesis. Different mitochondrial DNA haplotypes, for example, were found among workers at the same collection site in *Z. nevadensis* (*40*) and *Reticulitermes* spp. (*44*) and *R. flavipes* (*24*). In colonies of *Nasutitermes corniger* with a small numbers of queens, the queens had different haplotypes and were thus maternally unrelated. In colonies with large numbers of reproductives, however, all reproductives shared the same haplotype, suggesting replacement of a single founder pair by several offspring recruited by their natal colony (*14*). Similarly, in *Nasutitermes nigriceps* and *Nasutitermes costalis* all individuals of a colony shared the same mitochondrial DNA haplotype (*15, 16*). In these cases it cannot be determined whether a single reproductive queen or multiple kings and queens from the same matriline (secondary replacement reproductives) reproduce in a colony.

To decide if a colony contains a single pair or has multiple reproductives, even if they stem from the same matriline, an allelic approach has to be employed. Using alloenzymes and microsatellite genotypes, one can test whether the distribution of the homo- and heterozygote genotypes in a colony deviate from the Mendelian ratios expected for a single pair (e.g., *15*).

Alloenzymes are appropriate for analyzing the colony social organization of termite species with sufficient genetic variability. In some termite species Mendelian distribution of genotypes in the majority of colonies show that monogamy is the predominant mode of reproduction. For example, alloenzymes have shown that colonies of *N. nigriceps* are headed predominantly by single unrelated pairs, which mate randomly. Colonies therefore show little inbreeding. Only in 7 in 136 colonies did alloenzymes show offspring genotype distribution indicating multiple reproductives (*15*). As mentioned above, individuals shared the same mitochondrial DNA haplotype, which confirms that multiple reproductives have developed within their natal colony. Similarly, colonies of *Incisitermes schwarzi* are generally simple genetic families headed by one reproductive pair (*45*). Yet, a quarter of the colonies in the field showed multiple related reproductives and sometimes the alloenzyme patterns of the

offspring were a mixture of the founder pair and the replacement breeders. This reveals the natural turnover in the colony cycle of many termite species, which prolongs a colony's life far beyond the lifespan of its founders. After the death of the founder pair in mature colonies, reproduction is continued by multiple replacement reproductives, which are offspring of the colony founders and therefore related (*Nasutitermes* spp.: *14*, *15*; *Hodotermopsis spp.*: *34*, *Reticulitermes* spp.: *9*, *33*).

For species with low alloenzyme variation genomic DNA has been employed to determine number and relatedness of breeders. Multilocus DNA fingerprinting has shown that about half of the colonies of *S. lamanianus* were headed by an unrelated pair, probably the colony founders, while the other colonies contained multiple related reproductives. Comparison of the degrees of relatedness among multiple breeders and their offspring to the expections assuming various numbers of generation turnovers suggests that only one inbreeding cycle took place (*46*).

Microsatellite genotypes show up to 50 alleles per locus and thus have a much higher resolution power than alloenzymes (*21*). To date, microsatellite primers have been developed for *Macrotermes michaelensi* (*29*), *R. flavipes* (*27*), *Coptotermes lacteus* (*30*) and *C. formosanus* (*28*), and studies on the social organization in most of these species are currently under way. First studies on the social organization of *C. formosanus* show an intriguing difference in the reproductive structure of colonies in introduced versus native populations. In introduced populations of Hawaii and Louisiana the proportion of colonies headed by multiple reproductives ranges from 36-65%. However, in a native population from Guangdong, China, all 14 investigated colonies were headed by multiple reproductives (Husseneder, Vargo, Grace, unpubl.).

The prime example of the possible variety of alternative breeding systems depending on species, location, or ecological factors are the *Reticulitermes* species in the USA and Europe. A number of studies employing alloenzymes and mitochondrial DNA have shown that colonies can be headed by single outbred pairs, multiple inbred reproductives, as well as multiple maternally unrelated breeders (*4*). Clément's enzymatic studies showed geographical variation in breeding systems for *Reticulitermes santonensis* and the *Reticulitermes lucifugus* species complex in Europe (*8*, *33*). Social organization in these species correlated with season and location. Living in humid climates with no resource limitation, colonies of *R. santonensis* and subspecies of *R. lucifugus* were generally highly polygynous. In contrast, in geographical regions with dry climate, monogyny combined with high aggression between colonies was prevalent. Alloenzymes and mitochondrial DNA studies on *R. flavipes* in the USA demonstrated considerable variation in colony and breeding structure with colonies headed by single unrelated pairs, colonies

containing multiple inbred replacement reproductives and large multiple nest colonies containing unrelated queens (*9, 24, 44*).

Molecular methods have so far revealed a wide range of possible breeding systems in termites. This reproductive plasticity facilitates adaptation to different ecological challenges and makes termites successful in establishing themselves in a variety of habitats (*2, 4*). On the one hand, colonies headed by single unrelated pairs are common. The fact that the majority of colony founders are unrelated suggests that mating with closely related nestmates during the process of swarming and colony foundation is limited. Shellman-Reeve (*20*) has provided the first evidence based on multilocus DNA fingerprinting that adults of *Z. nevadensis* actively avoid pairing with nestmates when given a choice.

On the other hand, colonies can be genetically heterogenous societies containing multiple reproductives with varying degree of relatedness. This can create structures within colonies. For example, in the African termite *S. lamanianus,* genetic differentiation was found between individuals from different collection sites of one single large colony. This suggests multiple nests with reproductives within a colony that are spatially segregated, yet still connected by worker exchange (*18*). In colonies containing multiple reproductives, termites from the same tunnel were genetically more similar than termites from different tunnels and termites within the nest. This suggests kin-biased foraging, i.e., termites segregate according to kin lines during foraging (*13*). We are currently investigating the possibility of such intracolonial structuring in several subterranean termite pest species. Separate breeding units and kin-biased foraging could influence penetration of a colony by bait toxin, and has implications for the effect of kin structure on cooperative behavior within spatially complex termite colonies.

Conclusion

The application of molecular markers to the study of termites is a fairly young science, and, until recently, has received little attention compared to research on ants, bees and wasps. Nevertheless, the use of molecular methods has already provided important new insights into many areas of termite biology, including population structure, colony identification and breeding systems of various termite species. Comparative research on a variety of termite species will bring us closer to understanding the genetic and ecological determinants of life in the colony, including conflict and cooperation, optimal levels of inbreeding and reproductive strategies. Understanding the constraints and possibilities in the life of termites will also help to control pest species.

References

1. Jones, S. C. In *Handbook of structural insect pests*; Gold, R. E.; Jones, S. C. Eds.; Entomol. Soc. America: Lanham, MD, **2000**, pp 119-124.
2. Shellman-Reeve, J. S. In *Social behaviour in insects and arachnids*; Choe, J. C.; Crespi, B. J., Eds.; Cambridge University Press: Cambridge, **1997**, pp 52-93.
3. Ross, K. G. *Mol. Ecol.* **2001**, *10*: 265-284.
4. Thorne, B. L.; Traniello, J. F. A.; Adams, E. S.; Bulmer, M. *Ethol. Ecol Evol.* **1999**, *11*, 149-169.
5. Pamilo, P.; Gertsch, P.; Thorén, P.; Seppä, P. *Annu. Rev. Ecol. Syst.* **1997**, *28*, 1-25.
6. Kamphampati, S.; Kjer, K. M.; Thorne, B. L. *Ins. Mol. Biol.* **1996**, *5*, 229-238.
7. Miura, T.; Maekawa, K.; Kitade, O.; Abe, T.; Matsumoto, T. *Ann. Entomol. Soc. Am.* **1998**, *91*, 515-523.
8. Clément, J.–L. *Sociobiology* **1986**, *11*, 311-323.
9. Reilly, L. M. *Am. Nat.* **1987**, *130*, 339-349.
10. Korman, A. K.; Pashley, D. P. *Sociobiology* **1991**, *19*, 41-50.
11. Strong, K. L.; Grace, J. K. *Pan-Pac. Entomol.* **1993**, *69*, 51-56.
12. Broughton, R. E.; Grace, J. K. *Sociobiology* **1994**, *24*, 121-126.
13. Kaib, M.; Husseneder, C.; Epplen, C.; Epplen, J. T.; Brandl, R. *Proc. Royal Soc. Lond. B.* **1996**, *263*, 1527-1532.
14. Atkinson, L.; Adams, E. S. *Proc. R. Soc. Lond. B.* **1997**, *264*, 1131-1136.
15. Thompson, G. J.; Hebert, P. D. N. *Heredity* **1998a**, *80*, 48-55.
16. Thompson, G. J.; Hebert, P. D. N. *Ins. Sociaux.* **1998b**, *45*, 289-299.
17. Husseneder, C. Ph.D. Diss., Univ. of Bayreuth, Bayreuth, Germany, **1998**.
18. Husseneder, C.; Kaib, M.; Epplen, C.; Epplen, J. T.; Brandl, R. *Mol. Ecol.* **1998**, *7*, 983-990.
19. Jenkins, T. M.; Basten, C. J.; Dean, R.; Mitchell, S. E.; Kresovich, S.; Forschler, B. T. *Sociobiology* **1999a**, *33*, 239-263.
20. Shellman-Reeve, J. S. *Anim. Behav.* **2001**, *61*, 869-876.
21. Parker, P. G.; Snow, A. A.; Schug, M. D.; Booton, G. C.; Fuerst, P. A. *Ecology* **1998**, *79*, 361-382.
22. Hoy, M. *Insect molecular genetics*. Academic Press: San Diego, CA, **1994**.
23. *Molecular Systematics*; Hillis, D. M.; Moritz, C., Eds.; Sinauer Associates, Inc.: Sunderland, MA, **1990**.
24. Bulmer, M. S.; Adams, E. S.; Traniello, J. F. A. *Behav. Ecol. Sociobiol.* **2001**, *49*, 236-243.

25. Simon, C.; Frati, F.; Beckenbach, A.; Crespi, B.; Liu, H.; Flook, P. *Ann. Entomol. Soc. Am.* **1994**, *87*, 651-701.
26. *Molecular tools for screening biodiversity;* Karp, A.; Isaac, P. G.; Ingram, D. S. Eds.; Chapman and Hall: London. **1998**.
27. Vargo, E. L. *Mol. Ecol.* **2000**, *9*, 817-820.
28. Vargo, E. L.; Henderson, G. *Mol. Ecol.* **2000**, *9*, 1935-1938.
29. Kaib, M.; Hacker, M.; Over, I.; Hardt, C.; Epplen, J. T.; Bagine, R. K. N.; Brandl, R. *Mol. Ecol.* **2000**, *9*, 502-504.
30. Thompson, G. J.; Lenz, M; Crozier, R. H. *Mol. Ecol.* **2000**, *9*, 1932-1934.
31. Husseneder, C.; Vargo, E. L.; Grace, J. K. Proc. XXI. Int. Congress Entomol. *Sociobiology* **2001**, in press.
32. Santos, O.; Luykx, P. *Biochem. Genet.* **1985**, *23*, 729-740.
33. Clément, J. L. In *Biosystematics of social insects;* Howse, P. E.; Clément, J. L., Eds.; Academic Press: London, **1981**; pp 49-61.
34. Wang, J.; Grace, J. K. *Hawaii Agriculture: Positioning for Growth. Conference Proceedings. CTAHR University of Hawaii, Honolulu.* **1995**, pp 168-169.
35. Korman, A. K.; Pashley, D. P.; Haverty, M. I.; La Fage, J. P. *Ann. Entomol. Soc. Am.* **1991**, *84*, 1-9.
36. Wang, J.; Grace, J. K. *Sociobiology* **2000a**, *36*, 21-31.
37. Reilly, L. M. Ph. D. Diss., Vanderbilt University, TN, **1986**.
38. Wang, J.; Grace, J. K. *Sociobiology* **2000b**, *36*, 7-19.
39. Wang, J., and J. K. Grace. *Sociobiology* **2000c**, *36*, 1-6.
40. Broughton, R. E. *Ann. Entomol. Soc. Am.* **1995**, *88*: 120-128.
41. Husseneder, C.; Grace, J. K. *IRG.* **2000**, WP 00-10371.
42. Husseneder, C.; Grace, J. K. *Env. Entomol.* **2001a**, *30*, 262-266.
43. Husseneder, C.; Grace, J. K. *J. Ins. Behav.* **2001b**, *14*, 173-186.
44. Jenkins, T. M.; Basten, C. J., Kresovich, S.; Forschler, B. T. *Sociobiology* **1999b**, *34*, 161-172.
45. Luykx, P. *Insectes Soc.* **1993**, *40*, 191-205.
46. Husseneder, C.; Kaib, M.; Epplen, C.; Epplen, J. T.; Brandl, R. *Behaviour* **1999**, *136*, 1045-1063.