Allozymic Relationships Among Cuticular Hydrocarbon Phenotypes of *Zootermopsis* Species (Isoptera: Termopsidae)

AMY K. KORMAN, DOROTHY P. PASHLEY,
MICHAEL I. HAVERTY,[1] AND JEFFERY P. LA FAGE

Department of Entomology, Louisiana State University,
Baton Rouge, Louisiana 70803

Ann. Entomol. Soc. Am. 84(1): 1–9 (1991)

ABSTRACT Genetic relationships among and within three species of *Zootermopsis* were examined using protein electrophoresis. Results were compared with four cuticular hydrocarbon phenotypes: *Z. angusticollis* (Hagen), *Z. laticeps* (Banks), *Z. nevadensis nevadensis* (Hagen), and *Z. nevadensis nuttingi* Haverty and Thorne. Allozyme data supported the position of *Z. angusticollis*, *Z. laticeps*, and *Z. nevadensis* as separate species and were consistent with cuticular hydrocarbon groupings. However, the two subspecies or cuticular hydrocarbon groupings within *Z. nevadensis* were not genetically distinct. If these subspecies are not interbreeding, divergence has been recent because genetic differentiation has not occurred. Further studies are required to determine the basis for the inconsistency between cuticular hydrocarbon patterns and allozymes within the *Z. nevadensis* complex.

KEY WORDS Insecta, *Zootermopsis*, speciation, chemosystematics

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PHENOTYPIC VARIATION associated with eusociality often confounds correct identification of species, especially in Isoptera. Characters generally used to distinguish termite species include morphological and ecological traits of the imago and soldier castes. As a result, several biochemical methods have been used to study systematic problems in social insects. These techniques include characterization of defensive secretions (Prestwich 1983), pheromones, and enzyme electrophoresis (Howse & Clément 1981). In addition, qualitative and quantitative differences in cuticular hydrocarbon components have been examined in a number of termite genera: *Reticulitermes* spp. (Howard et al. 1978, 1980, 1982b; Clément et al. 1986), *Nasutitermes* spp. (Moore 1969; Howard et al. 1988; M.I.H. et al., unpublished data), *Zootermopsis* spp. (Blomquist et al. 1979, Haverty et al. 1988), and *Coptotermes formosanus* Shiraki (Haverty et al. 1990). Differences in hydrocarbon composition among many species of termites have been so striking that they have been suggested to function as cues for species and caste recognition (Howard et al. 1982b, Howard & Blomquist 1982).

One termite genus currently under investigation for new taxonomic characters is *Zootermopsis*, a group of primitive dampwood termites restricted to western North America. The most recent taxonomic studies of this genus, conducted >50 yr ago (Emerson 1933, Sumner 1933), recognized three extant species: *Z. nevadensis* (Hagen), *Z. angusticollis* (Hagen), and *Z. laticeps* (Banks). Imagines and soldiers of *Z. laticeps* are easily separated from *Z. angusticollis* and *Z. nevadensis* by the shape of the lateral margins of the pronotum. In the imago, the latter two species are distinguished primarily by eye size and color; mandible length and head shape separate soldiers (Sumner 1933). However, species diagnoses based on these characters are often unreliable. Thorne & Haverty (1989) have shown that a mandibular character (shape and position of the subsidiary tooth) can be used to separate all extant species of *Zootermopsis* unequivocally.

Geographically, *Z. nevadensis* and *Z. angusticollis* are distributed sympatrically along the Pacific Coast from Baja California, Mexico, to British Columbia, Canada. However, *Z. nevadensis* extends farther eastward into the continent, at least as far as western Montana (Weesner 1965; B. L. Thorne & M.I.H., unpublished data). *Z. laticeps* is found along waterways in southern New Mexico and Arizona southward through the Sonoran and Chihuahuan deserts into Mexico (Weesner 1965). Its range does not overlap with those of either *Z. nevadensis* or *Z. angusticollis*. *Z. angusticollis* and *Z. nevadensis* colonize dead wood, particularly that of the common conifers *Pseudotsuga* and *Pinus*, whereas *Z. laticeps* infests only the dead, central portions of trunks and large branches of willow, cottonwood, and sycamore.

Haverty et al. (1988) identified four unique cuticular hydrocarbon phenotypes in *Zootermopsis*. Two of these were exclusive (and unique within species) to *Z. angusticollis* (phenotype II) and *Z. laticeps* (phenotype IV). The other two phenotypes were observed in *Z. nevadensis* (phenotypes I and

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[1] Pacific Southwest Research Station, Forest Service, USDA, P.O. Box 245, Berkeley, Calif. 94701.

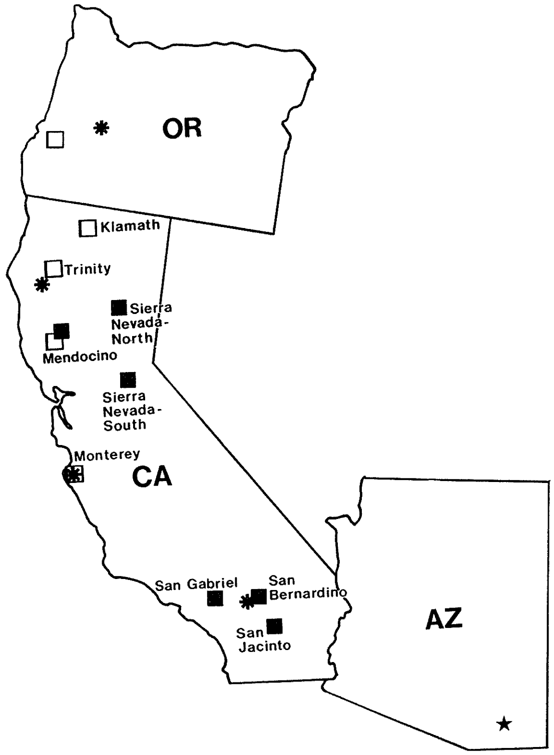

Fig. 1. Geographic distribution of populations of *Zootermopsis* species designated by cuticular hydrocarbon phenotypes (Haverty et al. 1988); *Z. nevadensis nevadensis* (■), *Z. nevadensis nuttingi* (□), *Z. angusticollis* (*), *Z. laticeps* (★).

III). Studies of agonistic behavior among the four cuticular hydrocarbon phenotypes were used to describe a new subspecies, *Z. nevadensis nuttingi* (Haverty & Thorne 1989). If cuticular hydrocarbon phenotypes are species-specific as suggested by Howard & Blomquist (1982), it follows that there may be at least one unrecognized species in this genus.

Because of the relatively high resolving power of electrophoresis in the discrimination among closely related sibling species (Avise 1975, Ayala 1975), we felt that *Zootermopsis* presented an excellent opportunity to determine genetic relationships among termite species within the genus while evaluating congruence between cuticular hydrocarbon phenotypes and allozymes.

Materials and Methods

To determine the extent of genetic variation throughout the range of *Zootermopsis*, collection efforts were directed to sampling colonies over a large geographic range as opposed to sampling colonies within a site. Specimens were obtained from 74 colonies that are designated as 16 geographic populations throughout Arizona, California, and Oregon (Fig. 1). Sample sites were chosen from various locations within each population. Three to 15 pseudergates (undifferentiated larvae of at least the third instar) per colony were collected for electrophoresis, transported live to Louisiana State University, and frozen at −70°C until they were electrophoresed. Seventy-three colonies were assigned to a cuticular hydrocarbon phenotype (by M.I.H.), and one colony sample of *Z. angusticollis* was assigned to species (and thus to cuticular hydrocarbon phenotype) using morphology.

Standard procedures for horizontal starch gel electrophoresis are found in Selander et al. (1971), Harris & Hopkinson (1976), and Richardson et al. (1986). Twenty-seven enzyme systems were surveyed in four gel electrode buffer systems (see Pashley [1983] for specific recipes). Activity was noted in 20 of these enzyme systems; 17 presumptive loci were resolved and scorable. One locus, mannose phosphate isomerase (monomorphic for *Z. nevadensis* and *Z. angusticollis*), was not resolved for *Z. laticeps*.

Individuals from different colonies within a population were pooled to calculate measures of genetic variability. Allele frequencies were determined for each population by first calculating colony allele frequency, then averaging across colonies. This was necessary because sample size varied, and pooling individuals among colonies gave increased weight to colonies with larger sample size and thus yielded inaccurate allele frequencies. Allele frequencies were used as BIOSYS-1 (Swofford & Selander 1981) input to generate Nei's (1972) and Rogers modified (Wright 1978) distance matrices. The unweighted pair group method of arithmetic averaging (UPGMA, Sneath & Sokal 1973) was used to cluster genetic distances between populations. A cophenetic correlation coefficient (Sokal & Rohlf 1962) indicated the goodness of fit of the original distance matrix to the phenogram. The integrity of the branch lengths and nodes of the phenogram was determined by jackknifing (Lanyon 1985) populations. This procedure allowed repeated construction of the phenogram after systematically eliminating one population from the input.

Voucher specimens of larvae, soldiers, and alates (when available) were deposited in the Museum of the Department of Entomology, Louisiana State University, Baton Rouge.

Results

Within *Zootermopsis*, 11 loci were polymorphic: aspartate aminotransferase (AAT), acid phosphatase (ACPH), alkaline phosphatase (APH), NADH diaphorase-2 (DIA), esterase-2 (EST), glucophosphoisomerase (GPI), glucose-6-phosphate dehydrogenase (G6PD), isocitrate dehydrogenase (IDH), peptidase (PEP), 6-phosphoglucose dehydrogenase (6PGD), and superoxide dismutase (SOD); 6 loci were monomorphic: fumarase, malic enzyme, malate dehydrogenase, mannose phosphate isomerase,

Table 1. Measures of genetic variation in *Zootermopsis* species

Species	No. loci	No. individuals/ locus, $\bar{x}$ ± SE	% Polymorphic loci[a]	No. alleles/ locus, $\bar{x}$ ± SE	Heterozygosity, $\bar{x}$ ± SE	
					Observed (direct count)	Expected (unbiased estimate)
Z. nevadensis	17	120.6 ± 8.5	29.4	1.9 ± 0.3	0.028 ± 0.016	0.080 ± 0.033
Z. angusticollis	17	35.2 ± 2.3	52.9	1.9 ± 0.3	0.076 ± 0.043	0.199 ± 0.055
Z. laticeps	16	20.2 ± 2.1	12.5	1.1 ± 0.1	0.009 ± 0.009	0.035 ± 0.027

[a] A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95.

phosphoglucomutase, and xanthine dehydrogenase. Allele frequencies, by population, are shown in Appendix A. Z. *angusticollis* exhibited the greatest genetic variability within the genus, and Z. *laticeps* was least variable. Heterozygosity values and other measures of genetic variability for the three species are shown in Table 1.

Both genetic distance measures calculated from 16 scorable loci in all species produced similar UPGMA phenograms. Rogers modified distance (matrix, Appendix B) produced a cluster analysis with the highest cophenetic correlation coefficient (0.98) (Fig. 2). The three major groupings within the phenogram correspond to the three species of *Zootermopsis* currently recognized.

Zootermopsis laticeps is the most genetically distinct of the three species and contains four diagnostic loci. One diagnostic locus is present in Z. *nevadensis,* and Z. *angusticollis* contains a nearly fixed difference for a unique allele at one locus. There were no fixed differences among Z. *nevadensis* populations.

In Z. *angusticollis,* geographic clusters of populations are apparent. Northern (Trinity and Oregon) and southern (Monterey and San Bernardino) populations cluster together. The two northern populations are located in the Coast and Cascade mountain ranges. These regions are more similar ecologically to each other than they are to the areas where the southern California populations (San Bernardino and Monterey) are located. The southern California sites are considerably more xeric than the areas sampled in northern California and Oregon. Large genetic distances between these regions suggest that populations have little, if any, gene exchange. Also, the high values within the two regions suggest gene flow may be restricted.

Within Z. *nevadensis,* cuticular hydrocarbon phenotypes (or subspecies) are not distinct genetic entities. The Klamath and Trinity populations, both of phenotype III (Z. *n. nuttingi*), cluster with the San Gabriel population (phenotype I, Z. *n. nevadensis*), whereas the three populations of phenotype III are clustered together on another branch. All subsequent phenograms produced by jackknifing had corresponding cophenetic correlation coefficients of at least 0.98. The only population of Z. *nevadensis* subject to a change in position was San Gabriel. When Klamath was eliminated, San Gabriel clustered with other phenotype I populations, and Trinity-III remained between this cluster and Mendocino-I. Likewise, when Sierra Nevada N-I was removed, San Gabriel moved into the top cluster with other populations of phenotype I, whereas Trinity and Klamath remained in place. Although this indicated some minor structural instability, in no case did the two populations of phenotype III (Klamath and Trinity) cluster with other phenotype III populations.

Discussion

Levels of Genetic Variation in *Zootermopsis*. Measures of genetic variability in *Zootermopsis* species are similar to values determined for other termites as well as to average values for insects. Clément (1981) found 52% of the loci to be polymorphic in European *Reticulitermes* species. Mean polymorphism for the class Insecta, summarized from published reports (Nevo 1978), is ≈40%. Mean heterozygosity (expected) for insects is 0.107 (Graur 1985), a value similar to that of *Zootermopsis*. Thus, variability within this genus is similar to that of many other groups of insects.

Observed values of heterozygosity for the three species, however, are considerably less than the expected values. Production of supplemental reproductives (neotenics) that mate with other siblings and enhance colony reproduction rate is common among termites. Therefore, differences in expected and observed heterozygosities are probably the result of the inbreeding (nonrandom mating between related individuals) within *Zootermopsis* colonies. Inbreeding may also be influenced by the asynchronous swarming of alates from the same nest relative to other colonies (Reilly 1987), which causes siblings to become the founding parents of a new colony.

Myles & Nutting (1988) summarized the tendency to produce neotenic reproductives in a number of termite families. Their information suggests that drywood termites (Kalotermitidae) are probably the least inbred of all termite families. Other termite genera (such as *Reticulitermes*) opportunely produce many neotenic reproductives within a colony. In *R. flavipes* (Kollar), Reilly (1987) showed that colonies were inbred and genetically isolated from neighboring colonies within the same locality. Within *Zootermopsis* species, inbreeding may be moderate and fall between that of the Kalotermitidae and *Reticulitermes*. Production of neotenics in this group is reported to be a result of

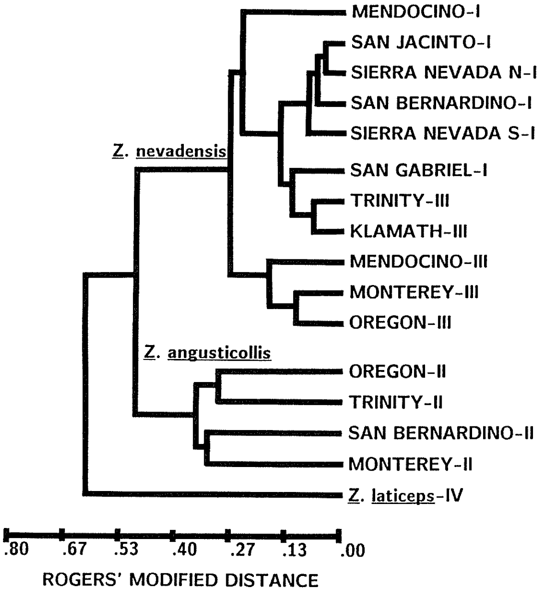

Fig. 2. UPGMA phenogram of distance relationships among *Zootermopsis* populations; cophenetic correlation coefficient = 0.98. Roman numerals indicate cuticular hydrocarbon phenotypes: I, Z. *nevadensis nevadensis;* III, Z. *n. nuttingi;* II, Z. *angusticollis;* IV, Z. *laticeps.*

reproductive stress, particularly orphaning (Myles & Nutting 1988). Colonies, however, may also enhance size and reproduction potential through fusion with adjacent colonies (Haverty & Thorne 1989), and the colonies that fuse might have been established by siblings.

Congruence Between Allozymes and Cuticular Hydrocarbon Analysis. As a major lipid class of surface waxes in insects (Hadley 1980), cuticular hydrocarbons provide protection against desiccation as well as a potential means of chemical communication within and among species (Howard & Blomquist 1982). Phenotyping using these lipids is based on quantitative or qualitative differences in hydrocarbon components (Howard et al. 1982b, 1988; Haverty et al. 1990).

In *Zootermopsis*, behavioral assays of soldiers, nymphs, and pseudergates showed that one cuticular hydrocarbon phenotype reacts agonistically to individuals of another phenotype (Haverty & Thorne 1989). Therefore, cuticular hydrocarbons are correlated with behavioral responses and may play a role in reproductive isolation between phenotypes (or species). The importance of cuticular hydrocarbons for species recognition has been further emphasized by the discovery that some termitophiles may be capable of mimicking the cuticular hydrocarbon composition of their host and thus gain acceptance into the colony (Howard et al. 1980, 1982a). Because hydrocarbon composition is unique among the few termite species that have been examined, it has been suggested that cuticular hydrocarbon profiles are species-specific and can be of considerable taxonomic value (Howard et al. 1978, Blomquist et al. 1979, Howard & Blomquist 1982). It might then follow that allozymes and cuticular hydrocarbon differentiation should show congruent patterns.

In *Zootermopsis,* congruence of electrophoretic data and cuticular hydrocarbon phenotypes is limited to species differences. Among *Zootermopsis* populations of the three recognized species, fixed or nearly fixed differences in allele frequencies support their current taxonomy. No fixed differences in allele frequencies or consistent distance relationships among populations existed between the two Z. *nevadensis* cuticular hydrocarbon phenotypes or subspecies.

There are two possible reasons these identification techniques are not congruent in Z. *nevadensis.* First, the composition of the cuticular hydrocarbons may be evolving faster than the allozymes. Toolson & Kuper-Simbrōn (1989) showed that significant quantitative change in hydrocarbon components and associated permeabilities can occur within an isofemale line of *Drosophila pseudoobscura* Frolova within a few generations. It is possible that the two cuticular hydrocarbon phenotypes in Z. *nevadensis* represent taxa in the very early stages of speciation, and allozymes do not yet reflect these reproductively isolated groups.

The second explanation is that cuticular hydrocarbon phenotypes are not indicative of evolutionary histories. For example, although Z. *laticeps* and Z. *n. nuttingi* are genetically dissimilar, they have cuticular hydrocarbon mixtures that are qualitatively more similar to each other than to Z. *angusticollis* or to the other subspecies, Z. *n. nevadensis.* It also has been shown in various arthropods that the quantity of these lipids can be affected by the environment (i.e., habitat, temperature, relative humidity, and season) (Hadley 1977, Toolson & Hadley 1979, Toolson 1982). Thus, cuticular hydrocarbon phenotypes in Z. *nevadensis* may be differentiating along some geographic cline in response to a selection gradient.

If environmental selection influences cuticular hydrocarbon composition, then phenotype II (Z. *angusticollis*) may be more environmentally plastic because it occurs across some of the same environmental gradients as Z. *nevadensis* yet exhibits only one cuticular hydrocarbon phenotype. Perhaps the key to understanding genetic relationships among Z. *nevadensis* populations lies in the climatological–geological history of the area they occupy.

Although there might be a close association between cuticular hydrocarbon phenotype and agonistic behavior, there is no direct evidence that cuticular hydrocarbons facilitate reproductive isolation as earlier suggested. Intraspecific agonistic behavior between larvae or imagines of *Zootermopsis* does not always prohibit reproductive interaction. Alates of phenotypes I and III have been known to pair and produce progeny under laboratory conditions (Haverty & Thorne 1989). In addition, Haverty & Thorne (1989) have noted considerable intraphenotypic aggression displayed toward primary and supplemental reproductives of different colonies. Aggressive behavior alone does not always indicate species isolation. Intraspecific aggression occurs among colonies of an ant, *Camponotus vagus* Scopoli, which possesses different proportions of cuticular hydrocarbons (Bonavita-Cougourdan et al. 1987). In termites, intraspecific aggression has been noted in *Coptotermes acinaciformes* (Froggatt) (Howick & Creffield 1980) and in *Coptotermes formosanus* Shiraki (Su & M.I.H., unpublished data) without any indication of reproductive isolation. The relationship between intercolony aggression and colony reproduction and fusion is not fully understood. Additional work in this area would aid in clarifying the association between cuticular hydrocarbon phenotypes and reproductive isolation.

Although our genetic data do not support two distinct taxa of *Z. nevadensis*, our results should not be interpreted to mean that cuticular hydrocarbon phenotyping has little taxonomic value. In fact, these data emphasize the need for future studies within *Zootermopsis* and perhaps other groups of Isoptera. In particular, extensive sampling and phenotypic–genetic studies of *Z. nevadensis* colonies of the Klamath and Cascade Mountains would be of considerable value because this area is the apparent border between the two *Z. nevadensis* phenotypes in northern California. Information provided by cuticular hydrocarbon composition, coupled with behavioral, morphological, and genetic studies, may provide insight into processes of evolution and speciation in social insects.

Acknowledgment

We thank the following people for their assistance in providing specimens for this study: P. M. Haverty, L. J. Nelson, W. L. Nutting, M. Page, R. H. Scheffrahn, and B. L. Thorne. We also acknowledge the financial support of the Louisiana Pest Control Association, Pi Chi Omega, and the Internal Competitive Grant Program of the Pacific Southwest Research Station.

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Received for publication 18 January 1990; accepted 24 July 1990.

Appendix A

Allele frequencies for polymorphic loci in *Zootermopsis* populations

Locus	Population[a]/Cuticular hydrocarbon phenotype[b]															
	1/I	2/I	3/I	4/I	5/I	6/I	7/III	8/III	9/III	10/III	11/III	12/II	13/II	14/II	15/II	16/IV
AAT *n*[c]	5	5	7	8	7	1	2	8	5	2	2	5	3	4	1	9
A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.286	0.0	0.0	0.0
B	1.0	0.800	1.0	1.0	1.0	1.0	0.875	0.875	0.935	0.459	1.0	1.0	0.714	1.0	0.500	0.093
C	0.0	0.100	0.0	0.0	0.0	0.0	0.125	0.042	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
D	0.0	0.100	0.0	0.0	0.0	0.0	0.0	0.083	0.065	0.541	0.0	0.0	0.0	0.0	0.500	0.907
ACPH *n*	5	5	7	8	7	1	2	8	5	2	2	5	3	4	1	9
A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.667	0.0	0.0	0.0
B	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.333	1.0	1.0	0.0
C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
APH *n*	5	4	1	3	7	1	2	10	4	1	2	4	3	4	1	9
A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
B	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0
DIA *n*	5	5	7	8	7	1	2	8	5	2	2	5	3	4	1	9
A	0.0	0.0	0.0	0.0	0.0	0.0	0.335	0.042	0.133	0.0	0.0	0.083	0.0	0.0	0.0	0.0
B	0.067	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.067	0.167	0.250	0.317	0.0	1.0	0.0	0.0
C	0.933	1.0	1.0	1.0	0.952	1.0	0.665	0.958	0.750	0.833	0.750	0.0	1.0	0.0	1.0	1.0
D	0.0	0.0	0.0	0.0	0.048	0.0	0.0	0.0	0.050	0.0	0.0	0.600	0.0	0.0	0.0	0.0
EST2 *n*	5	5	7	8	7	1	2	8	5	2	2	5	3	4	1	9
A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.025	0.0	0.0	0.0	0.0
B	0.100	0.0	0.0	0.0	0.0	0.0	0.0	0.028	0.0	0.333	0.0	0.0	0.167	0.0	0.0	0.0
C	0.473	0.700	0.571	0.750	0.679	1.0	1.0	0.889	1.0	0.667	1.0	0.547	0.278	0.475	0.333	1.0
D	0.050	0.0	0.071	0.250	0.071	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E	0.0	0.0	0.0	0.0	0.036	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F	0.377	0.300	0.357	0.0	0.214	0.0	0.0	0.083	0.0	0.0	0.0	0.428	0.556	0.525	0.667	0.0

Appendix A
Continued

Locus	Population[a]/Cuticular hydrocarbon phenotype[b]															
	1/I	2/I	3/I	4/I	5/I	6/I	7/III	8/III	9/III	10/III	11/III	12/II	13/II	14/II	15/II	16/IV
GPI *n*	4	5	7	8	7	1	2	7	5	1	2	4	3	4	1	9
A	0.063	0.0	0.0	0.0	0.0	0.500	1.0	0.429	0.275	1.0	1.0	0.625	1.0	1.0	1.0	1.0
B	0.938	1.0	1.0	1.0	0.893	0.500	0.0	0.571	0.725	0.0	0.0	0.375	0.0	0.0	0.0	0.0
C	0.0	0.0	0.0	0.0	0.107	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
G6PD *n*	5	5	7	8	7	1	2	6	5	2	2	5	3	4	1	9
A	0.557	0.767	0.571	1.0	0.643	0.0	1.0	0.972	0.975	1.0	1.0	0.340	0.662	1.0	0.500	0.0
B	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.028	0.025	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C	0.443	0.233	0.429	0.0	0.357	0.0	0.0	0.0	0.0	0.0	0.0	0.660	0.378	0.0	0.500	0.0
D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
IDH *n*	5	5	7	8	7	1	2	8	5	2	2	5	3	4	1	9
A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.889	0.438	0.0	0.0
B	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	0.111	0.562	1.0	0.0
C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.278
D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.722
PEP *n*	5	5	7	8	7	1	2	8	5	2	2	5	3	4	1	9
A	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0
B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0	1.0
6PGD *n*	5	5	7	8	7	1	2	8	5	2	2	5	3	4	1	9
A	1.0	0.967	1.0	0.938	0.952	1.0	1.0	1.0	1.0	1.0	0.750	0.967	0.756	1.0	0.750	1.0
B	0.0	0.0	0.0	0.062	0.024	0.0	0.0	0.0	0.0	0.0	0.250	0.0	0.122	0.0	0.250	0.0
C	0.0	0.033	0.0	0.0	0.024	0.0	0.0	0.0	0.0	0.0	0.0	0.033	0.122	0.0	0.0	0.0
SOD *n*	5	5	7	8	7	1	2	8	5	2	2	5	3	4	1	9
A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.667	1.0	1.0	0.0
B	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	0.333	0.0	0.0	1.0

[a] *Z. nevadensis:* 1, Sierra Nevada North; 2, Sierra Nevada South; 3, San Jacinto; 4, San Gabriel; 5, San Bernardino; 6, Mendocino; 7, Monterey; 8, Trinity; 9, Klamath; 10, Mendocino; 11, Oregon. *Z. angusticollis:* 12, Trinity; 13, San Bernardino; 14, Oregon; 15, Monterey. *Z. laticeps*, 16, southeastern Arizona.

[b] Populations 1–11 are *Z. nevadensis* (*Z. n. nevadensis*, phenotype I; *Z. n. nuttingi*, phenotype III. Populations 12–15 are *Z. angusticollis* (phenotype II), and population 16 is *Z. laticeps* (phenotype IV).

[c] *n*, number of colonies sampled within population.

Appendix B

Distance matrix (Rogers' modified distance) of *Zootermopsis* populations

Population[a]	Population															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 Sierra Nevada N-I	—	0.086	0.034	0.146	0.062	0.212	0.296	0.171	0.173	0.306	0.294	0.502	0.483	0.510	0.451	0.636
2 Sierra Nevada S-I		—	0.072	0.101	0.065	0.245	0.281	0.133	0.131	0.290	0.284	0.522	0.495	0.520	0.460	0.627
3 San Jacinto-I			—	0.133	0.048	0.214	0.303	0.170	0.169	0.319	0.303	0.511	0.493	0.524	0.461	0.639
4 San Gabriel-I				—	0.107	0.287	0.273	0.124	0.109	0.297	0.269	0.542	0.514	0.525	0.492	0.647
5 San Bernardino-I					—	0.210	0.277	0.141	0.135	0.299	0.275	0.508	0.491	0.516	0.460	0.631
6 Mendocino-I						—	0.293	0.250	0.259	0.324	0.293	0.504	0.494	0.543	0.451	0.606
7 Monterey-III							—	0.164	0.188	0.165	0.103	0.517	0.465	0.451	0.438	0.594
8 Trinity-III								—	0.064	0.196	0.171	0.517	0.470	0.479	0.441	0.600
9 Klamath-III									—	0.235	0.197	0.513	0.494	0.482	0.468	0.615
10 Mendocino-III										—	0.172	0.534	0.457	0.460	0.409	0.563
11 Oregon-III											—	0.518	0.461	0.440	0.434	0.599
12 Trinity-II												—	0.327	0.287	0.376	0.636
13 San Bernardino-II													—	0.358	0.316	0.550
14 Oregon-II														—	0.333	0.645
15 Monterey-II															—	0.571
16 Z. *laticeps*-IV																—

[a] Roman numerals indicate cuticular hydrocarbon phenotype. Populations 1–11 are Z. *nevadensis*, 12–15 are Z. *angusticollis*, and 16 is Z. *laticeps*.