

Desiccation resistance in arboreal and terrestrial ants

W. GREGORY HOOD and WALTER R. TSCHINKEL Department of Biological Science, The Florida State University, Tallahassee, Florida, U.S.A.

ABSTRACT. Arboreal and terrestrial ants were exposed to 0, 25, 50, 75 and 100 (control)% r.h., at 30°C. Desiccation resistance increased with body size (as dry weight^{0.55}), but not as quickly as expected from the consequences of the surface area and volume relationship (as dry weight^{0.67}). Arboreal ants took 8 times longer to die than terrestrial ants of comparable size. Even after size effects were removed, desiccation resistance differed between various terrestrial species and showed a correlation with foraging patterns.

Arboreal and terrestrial ants whose waterproofing epicuticular lipids were removed by chloroform:methanol extraction had equally high water loss rates at 0% r.h. Unextracted arboreal ants had water loss rates half those of unextracted terrestrial ants, suggesting that differences between them were based on differences in epicuticular lipids. The lower water loss rates of arboreal ants contributed significantly to their longer survival under desiccation. Arboreal ants also had greater total rectal pad area than terrestrial ants, suggesting that they may be able to reclaim faecal water more effectively. There were no differences in the minimum viable water content between the two groups of ants. Both had water loss tolerances comparable with those of arthropods adapted to xeric environments. Initial water loss rates could not account for all of the differences in desiccation resistance between arboreal and terrestrial ants. Other adaptations to desiccation stress by arboreal ants are likely.

Comparisons of water loss rates and desiccation resistance between arboreal and desert ants suggest that the arboreal habitat is at least as stressful as the desert habitat.

Key words. Formicidae, arboreal ants, humidity, desiccation, water loss, epicuticular lipids, rectal pads.

Introduction

By excavating deep nests, ground-dwelling ants are capable of taking advantage of a vertical gra-

dient of temperature and humidity in the soil. During seasonal extremes in weather these ants can retreat to the comparatively invariant depths of the nest (Wilson, 1971). Arboreal ants, which nest in hollow reeds, vines, branches, bark or other exposed sites have no such refuge from inclement weather. Thus, one would expect that

Correspondence: Dr W. R. Tschinkel, Department of Biological Science, The Florida State University, Tallahassee, Florida 32306, U.S.A.

the distribution of arboreal ants be limited to areas of benign climate or that arboreal ants have special physiological adaptations to their demanding circumstances. There is evidence for both. Most ants in the temperate zone nest in the ground while in the tropics most ants nest in rotting wood, a smaller number are arboreal, and still fewer nest entirely in the ground (Wilson, 1971). Those few ants in temperate areas which nest in relatively exposed situations, such as arboreal ants, appear to have physiological adaptations to low humidity (Gösswald, 1938) and are likely to have adaptations to temperature extremes as well.

Water conservation is an important problem for terrestrial organisms, especially for those animals which are smallest. This is true because evaporative water loss is a function of surface area, and surface area relative to volume is greater in small organisms than in large. Thus water loss relative to water content is greater in small organisms than in large. One might expect adaptations for water conservation to be greatest in small animals that live in xeric circumstances. Thus, comparisons of insects from different environments offer dramatic examples of adaptation to water conservation.

Adaptations of arthropods to desiccating conditions include: body morphologies which minimize the surface area to volume ratio; lifestyles minimizing exposure to vapour pressure deficits; nutritional modes maximizing water gain and minimizing loss; controlled spiracular activity; specialized lipid-covered cuticles; efficient rectal reclamation of water and active water sorption; the production of metabolic water; and water loss tolerance (Hadley, 1974; Arlian & Veselica, 1979).

Myrmecologists have paid relatively little attention to the importance of humidity to ants. Much more attention has focused on the importance of temperature, probably because temperature is more easily measured and controlled. Some investigators have probed the water balance of desert ants (Ettershank & Whitford, 1973; Kay & Whitford, 1975; Whitford *et al.*, 1975) and non-desert terrestrial ants (Sigal & Arlian, 1982; Potts *et al.*, 1984; Coenen-Stass, 1986). However, except for Gösswald (1938) and Delyé (1968), few comparisons have been made between groups of ants from different environmental circumstances.

Our study compared the ability of a variety of

arboreal and terrestrial ants to resist desiccation-induced mortality. The contributions of several possible adaptations for water conservation were investigated, including comparisons of water loss tolerance, rectal pad number and scaling, and cuticular waterproofing.

Materials and Methods

Most of the ants used in the experiments were collected in Tallahassee, Florida, or in the nearby Apalachicola National Forest. One species (*Pseudomyrma mexicana* (Roger)) was collected from Miami, Florida. The desert ants were collected from an area 5 miles southwest of Rodeo, New Mexico. All ants collected in the Tallahassee area were subjected to experimental treatments on the day of their collection. Those from Miami or New Mexico were exposed to experimental conditions 3 or 4 days after collection. They were refrigerated during the interim. Collections were made during all but the winter months, late November through to March. James Trager provided identifications of the ant species. The arboreal ants in this study nested either in the dry, flaky bark of longleaf pine trees (*Pinus palustris*) or in the hollow stems of dead weeds and vines. The terrestrial ants nested in the soil and in rotting logs. Only workers were used.

Desiccation resistance

The relative vulnerability of arboreal and terrestrial ants to desiccation was determined by exposing groups of ants of various species to relative humidities (r.h.) of 100, 75, 50, 25 and 0% at 30°C. Periodic counts of live and dead ants were made and the time required to kill 50% of the ants in a group (LT₅₀) was calculated using the toxicological HANK computer program, acquired from the Florida Department of Environmental Regulation. The program uses probit analysis to calculate LT₅₀s. Generally, ants were collected from one colony and divided into five groups of ten to twenty-two ants, depending on the size of the colonies. Each group was then exposed to one of the five test humidities. However, species which had small colonies sometimes supplied only two groups of ants per colony. These were assigned to treatments of 100% and 0% r.h. Larger colonies provided

additional groups for the 50%, and sometimes the 25% r.h. treatments as well.

The desiccation apparatus consisted of a squat glass jar (c. 400 ml) containing either anhydrous CaCl_2 (0% r.h.), various concentrations of aqueous sulphuric acid (15, 50 and 75% r.h.), or water (100% r.h.). Four small vials were attached to the lid, each containing a group of ants which were prevented by copper screening from entering the large chamber. Ants were briefly chilled till immobile in order to place them easily in the small chambers. Preliminary tests indicated that there was no bias among various copies of the desiccation apparatus.

Ants used in the LT_{50} experiments were oven dried for a week at 60°C after their ordeal and weighed to the nearest 0.01 mg on a Mettler M55A balance or Cahn model 7500 millibalance. Voucher specimens of the ants used in these experiments were deposited in the Florida State Collection of Arthropods, Gainesville.

Statistical analysis

Unless otherwise stated, data were subjected to analysis of covariance (ANCOVA) on log transformed data. Adjusted means were compared at a 5% significance level using Bryant & Paulson (1976) *a posteriori* pairwise comparisons. Residual analysis was routinely performed to check for outliers and to determine if assumptions of normality and uniform variance held. When these assumptions were not warranted non-parametric analysis was used.

Results

Desiccation resistance

The length of time an ant survived under desiccating conditions increased with body size and relative humidity, and depended strongly on whether the species was arboreal or terrestrial (species type), as well as on the identity of the species within each type (Figs 1 and 2; Table 1). Species type had the greatest effect on LT_{50} , accounting for 59% of the variation in LT_{50} . Size explained 13% of the variation; humidity and species identity explained 9% each.

Larger ants had a smaller proportionate water loss rate, and therefore survived longer under

desiccating conditions (ANCOVA, $F=241.4$, d.f.=1, 102, $P<0.001$) (Fig. 1). Over a 100-fold range of weight, terrestrial ant LT_{50} s increased about 20-fold from a mean of 1.5 h to c. 30 h. For the 25-fold range of arboreal ant weights, LT_{50} s increased 7-fold c. 10 to 70 h.

The effect of size on LT_{50} is a consequence of the surface law: surface areas (A) of geometrically similar objects increase with volume^{2/3} ($V^{2/3}$). LT_{50} should vary with weight^{2/3} ($W^{2/3}$), assuming LT_{50} is dependent on water loss rate, water loss rate is dependent on surface area, and density is constant. Instead, the allometric relationship between dry weight and LT_{50} is described by the power function: $\text{LT}_{50}=kW^{0.546}$. This equation becomes linear when log transformed ($\log \text{LT}_{50}=\log k+0.546 \log W$). The effect of size was similar for each combination of humidity and species type – the regression coefficients of each were similar ($F=0.58$, d.f.=3, 102, $P<0.20$), hence the common regression coefficient, 0.546. The expected regression coefficient, 0.667, differed from the observed value of 0.546 ($t=3.59$, d.f.=102, $P<0.01$), indicating that the surface/volume relationship alone does not explain the effects of size on LT_{50} . LT_{50} s increased with size, indicating that either large ants had lower LT_{50} s than expected, or small ants higher, or both. Possible scaling of density, geometry, surface properties, or other adaptations to water stress with size could account for the deviation from the theoretical expectation. Small ants may be compensated for their high surface area to volume ratios with some relatively inexpensive adaptations to water stress. However, adaptations resulting in LT_{50} s comparable to those of large ants may be too expensive to be feasible. Conversely, large ants may have reduced adaptations to water stress and fewer associated costs.

The y axis intercept of the allometric equations (or the treatment effect when size is 1.0 mg) was higher for arboreal than terrestrial species and increased with humidity (Table 2). Arboreal ants survived exposure to low humidity an average of 8 times as long as terrestrial ants of similar size (ANCOVA, $F=478.4$, d.f.=1, 102, $P<0.001$). Indeed, the differences between the two groups of ants were so dramatic that some arboreal ants exposed to 0% r.h. survived longer than did any of the terrestrial ants exposed to 75% r.h. Somewhat surprisingly, Chihuahuan desert ants were no more desicca-

TABLE 1. Species used in LT₅₀ comparisons and rectal pad measurements.

Code	Division	Habitat	No. of rectal pads	Notes	No. of replicates (colonies) at each humidity level			
					0%	25%	50%	75%
Myrmecoid Complex								
Subfamily Pseudomyrmecinae								
8	<i>Pseudomyrma ejecta</i> F. Smith	Arboreal	—		2	—	2	—
9	<i>P. mexicana</i> Roger	Arboreal	—		3	—	3	—
10	<i>P. pallida</i> F. Smith	Arboreal	6		3	—	3	—
11	<i>P. seminole</i> Ward	Arboreal	6		1	—	1	—
Subfamily Dolichoderinae								
Tribe Dolichoderini								
d	<i>Dolichoderus mariae</i> Forel	Terrestrial	—		1	1	1	—
Tribe Tapinomini								
—	<i>Forelius pruinosus</i> Roger	Terrestrial	3		—	—	—	—
c	<i>Conomyrma</i> sp.	Terrestrial	3		3	3	3	3
Subfamily Formicinae								
Tribe Formicini								
e	<i>Formica pallidefulva</i> Mayr	Terrestrial	6		1	1	1	—
C	<i>F. perpilosa</i> Wheeler	Desert*	—		1	—	—	—
m	<i>Prenolepis imparis</i> (Say)	Terrestrial	2		2	—	1	—
l	<i>Brachymyrmex</i> sp.	Arboreal	2		3	4	4	3
h	<i>Paratrechina faisonensis</i> (Forel)	Terrestrial	—		5	5	5	3
j	<i>P. phantasma</i> Trager	Terrestrial	—		2	—	2	—
Tribe Camponotini								
b	<i>Camponotus floridanus</i> (Buckley)	Terrestrial	6	Majors	3	2	2	—
				Minors	3	2	2	1
2	<i>Camponotus nearcticus</i> Emery	Arboreal	6		2	2	2	2
3	<i>Colobopsis impressus</i> (Roger)	Arboreal	6		2	2	2	2
Poneroid Complex								
Subfamily Ponerinae								
Tribe Odontomachini								
9	<i>Odontomachus brunneus</i> Patton	Terrestrial	—		3	2	3	1
Subfamily Dorylinae								
Tribe Ecitoni								
f	<i>Neivamyrmex opacithorax</i> (Emery)	Terrestrial	2	Majors	1	1	1	—
				Minors	1	—	1	—
Subfamily Myrmicinae								
Tribe Myrmicini								
A	<i>Apaenogaster cockereilli</i> (E. Andre)	Desert	—		1	—	—	—
B	<i>A. cockereilli</i> as <i>Novomessor cockereilli</i>	Desert*	—		1	—	—	—
a	<i>A. floridana</i> M. R. Smith	Terrestrial	—		3	3	3	3
6	<i>Leptothorax wheeleri</i> (M. R. Smith)	Arboreal	3		5	3	5	2
7	<i>Monomorium trageri</i> DuBois	Arboreal	—		1	1	1	1
D	<i>Pheidole desertorum</i> Wheeler	Desert	—		1	—	—	—
k	<i>Pogonomyrmex badius</i> (Latreille)	Terrestrial	3		5	5	3	2
E	<i>P. barbatus</i> (F. Smith)	Desert*	—		1	—	—	—
F	<i>P. californicus</i> (Buckley)	Desert*	—		1	—	—	—
G	<i>P. desertorum</i> Wheeler	Desert	—		1	—	—	—
H	<i>P. desertorum</i>	Desert*	—		1	—	—	—
I	<i>P. rugosus</i> Emery	Desert*	—		1	—	—	—
J	<i>Solenopsis amblychila</i> Wheeler	Desert	—		1	—	—	—
n	<i>S. geminata</i> (Fabricius)	Terrestrial	—	0.81†	1	—	1	—
				0.39	2	1	2	1
				0.25	2	1	2	2
				0.17	5	4	5	5
p	<i>S. invicta</i> Buren	Terrestrial	3	1.45	1	—	1	—
				0.78	3	3	3	3
				0.49	3	3	3	2
				0.19	3	3	3	2
Tribe Crematogastrini								
4	<i>Crematogaster</i> n.sp. near <i>ashmeadi</i>	Arboreal	3		4	3	3	4
5	<i>C. clara</i> Mayr	Arboreal	—		3	3	3	3
Tribe Attini								
K	<i>Trachymyrmex desertorum</i> (Wheeler)	Desert	—		1	—	—	—
q	<i>T. septentrionalis</i> (McCook)	Terrestrial	3		3	3	3	3

*From Whitford *et al.* (1975) and Kay & Whitford (1975).

†Mean dry weight in mg.

tion resistant than other terrestrial ants (Fig. 1). For all three groups of ants only those succumbing to fungal infections or other unrelated causes died from the exposure to 100% r.h.

Within each species type there were differences in LT_{50} between species even after the effects of size were removed. The two fire ant species, *Solenopsis invicta* Buren and *S.geminata* (F.) (n and p, respectively, in Fig. 1), have

disjunct distributions in North Florida (Tschinkel, 1988a). An *a priori* comparison detected significant differences in their size-adjusted LT_{50} s ($F=4.59$, d.f.=8, 38, $P<0.05$). *S.invicta* had LT_{50} s approximately 1.3 times those of *S.geminata*, suggesting a correlation between their distribution and their desiccation resistance. *A posteriori* pairwise comparisons were made of all replicated terrestrial and arboreal species.

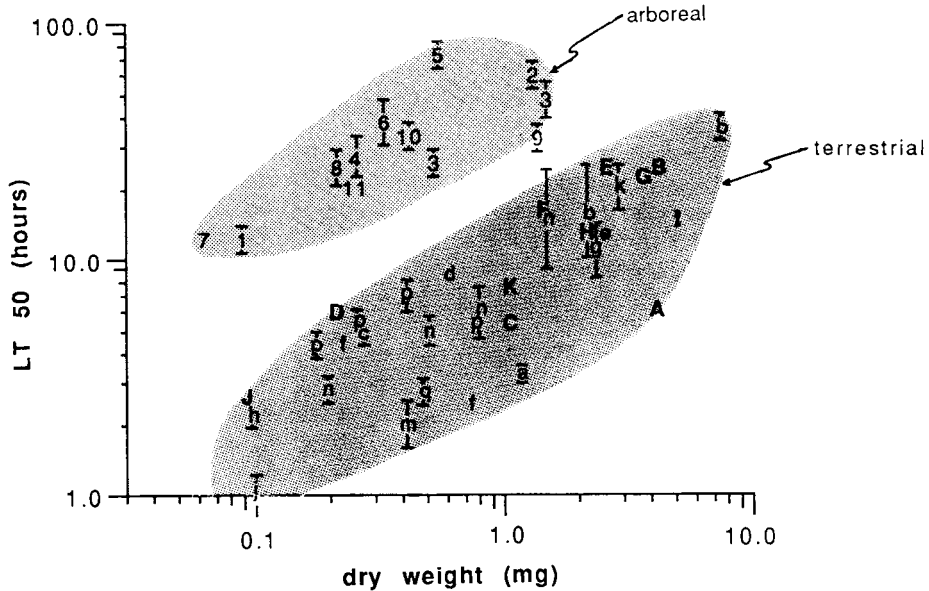


FIG. 1. LT_{50} s of arboreal, terrestrial and Chihuahuan ant species at 0% r.h. (log scale). Numbers or letters represent species and correspond to those in Table 1. Numbers identify arboreal species, lower case letters, terrestrial species, and upper case letters, Chihuahuan species. Each point represents the average LT_{50} for n colonies of each species. The number of replicates (n) is also listed in Table 1. Error bars are standard deviations. Shaded areas include all points representing either arboreal or terrestrial species.

TABLE 2. Regression equations of $\log LT_{50}$ versus \log dry weight, the standard deviations (SD) of slopes and intercepts, and the standard error of estimate (SE) for each species type at experimental relative humidities.

Species type	Relative humidity (%)	n	Slope	SD of slope	Intercept	SD of intercept	R^{2*}	SE
Terrestrial	0	22	0.609	0.094	0.890	0.051	0.661	0.217
	25	17	0.527	0.103	0.992	0.055	0.609	0.218
	50	22	0.580	0.061	1.135	0.033	0.812	0.144
	75	13	0.563	0.099	1.348	0.054	0.723	0.167
Arboreal	0	12	0.445	0.099	1.678	0.061	0.634	0.147
	25	7	0.527	0.098	1.875	0.070	0.824	0.116
	50	12	0.387	0.101	1.835	0.063	0.552	0.155
	75	6	0.653	0.186	2.268	0.142	0.693	0.213
Desert	0	11	0.443	0.123	0.957	0.069	0.543	0.221

* Adjusted for degrees of freedom.

Among arboreal species, *Crematogaster clara* Mayr (no. 5 in Fig. 1) had higher size-adjusted LT_{50} s than any other arboreal species except *Leptothorax wheeleri* M. R. Smith. Among the terrestrial ants, however, there were three categories: (1) species with relatively high size-adjusted LT_{50} s (*Solenopsis invicta*, *Pogonomyrmex badius* (Latreille), *Conomyrma* sp., *Camponotus floridanus* (Buckley) and *Paratrechina faisonensis* (Forel)); (2) species with intermediate LT_{50} s (*S. geminata*, *Odontomachus brunneus* Patton and *Paratrechina phantasma* Trager); and (3) species with relatively low LT_{50} s (*Trachymyrmex septentrionalis* (McCook), *Prenolepis imparis* (Say) and *Aphaenogaster floridana* M. R. Smith). A species was classified as intermediate if, after the effects of size were removed, its LT_{50} did not differ significantly ($P < 0.05$) from the LT_{50} s of at least eight of the ten species with whom it was compared. All species having high LT_{50} s differed significantly from all of those having low LT_{50} s, and had LT_{50} s almost 3 times as large. LT_{50} s of intermediate ants differed by a factor of 1.7 from each of the other groups.

Increased relative humidity increased survival of arboreal and terrestrial ants in a similar fashion ($F = 26.4$, d.f. = 3, 102, $P < 0.001$) (Fig. 2) with a common slope of 0.00536 (ANOVA of slopes; $F = 1.045$, d.f. = 12, 26, $P < 0.20$). An increase of 1% r.h. caused a 1.012-fold (anti-log of 0.00536) increase in LT_{50} , so that an increase of 25% r.h. caused a 1.4-fold increase (anti-log of 25×0.00536) in LT_{50} . When the effect of size was removed, the correlation (R^2) between LT_{50} and humidity was 90% for arboreal ants and 96% for terrestrial ants.

Water loss tolerance

One of the most widespread adaptations to arid environments is the ability to tolerate water loss during times of extraordinary drought (Hadley, 1972). Droughts are a fact of arboreal life with a severe impact on arboreal ants. Arboreal ants might therefore be more capable of tolerating water loss. If this is the case, the observed differences in LT_{50} s between arboreal and terrestrial ants might simply reflect different water loss tolerances.

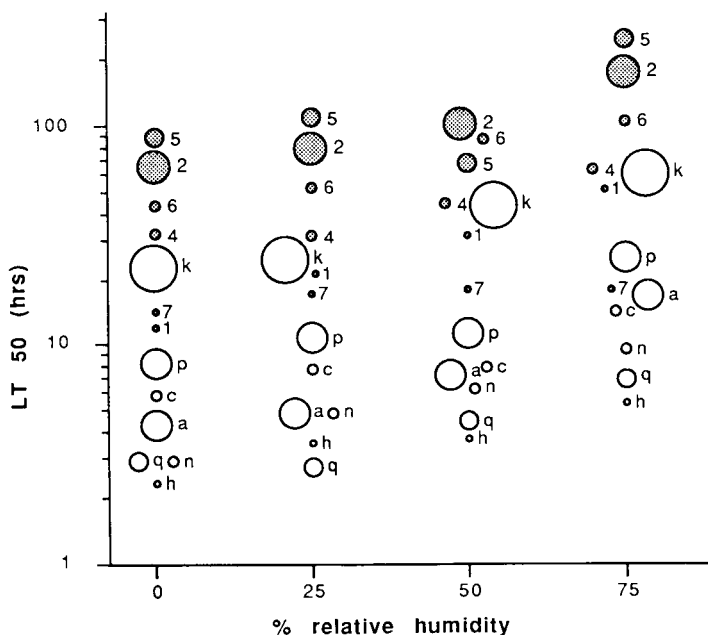


FIG. 2. Average LT_{50} s (log scale) of selected ants species at 0, 25, 50 and 75 r.h. Shaded and unshaded circles represent arboreal and terrestrial species respectively. Circles of increasing size represent size ranges of 0.06–0.09, 0.19–0.32, 0.39–0.52, 1.15–1.29 and 2.70–3.00 mg dry weight respectively. Numbers and letters, adjacent to circles, correspond to species codes in Table 1. None of the slopes of the species–humidity regressions were significantly different from any others ($F = 1.045$, d.f. = 12, 26, $P > 0.20$).

The ability of arboreal and terrestrial ants to tolerate water loss was determined by exposing individual ants to 0% r.h. at 30°C and gravimetrically calculating the amount of water lost from the start of the experiment to the moment an individual became incapable of normal locomotion. This was considered a functional equivalent of death. Ants were briefly chilled till immobile, weighed individually to the nearest 0.01 mg, weighed again when their locomotion was impaired, and finally weighed after being oven dried for a week. Three arboreal and three terrestrial species were used in this study (arboreal: *Pseudomyrma pallida* F. Smith, *Leptothorax wheeleri*, *Crematogaster* sp.; terrestrial: *Solenopsis invicta*, *Trachymyrmex septentrionalis*, *Conomyrma* sp.). Measurements were made on five ants in each of three colonies per species. All species consisted of ants of a similar size. The percentage of body water lost at death ($[(\text{initial wt} - \text{end wt}) / (\text{initial wt} - \text{dry wt})] \times 100$), the initial percentage water content ($[(\text{initial wt} - \text{dry wt}) / (\text{initial wt})] \times 100$), and the percentage water content at death ($[(\text{end wt} - \text{dry wt}) / (\text{end wt})] \times 100$) were compared.

There were no significant differences in water loss tolerance between the six species (Kruskal-Wallis, $H=9.80$, $P>0.08$). Water loss tolerance range from 40% to 54%. Although there were species differences in initial water content and water content at death there was no pattern of

differences corresponding to the arboreal/terrestrial dichotomy (Kruskal-Wallis, $P<0.025$ for both). Initial water content ranged from 60% to 70% and water content at death from 43% to 60%. Water loss tolerance is evidently not a major contributing factor to LT_{50} differences between arboreal and terrestrial ants.

Rectal pad allometry

Rectal pads are involved in the reabsorption of water from the faeces. If arboreal ants have more or larger rectal pads, these may reduce faecal water loss more than those of terrestrial ants.

Recta were dissected in 70% ethanol from four ants per colony, two colonies per species, for various species (Table 1). The length and width of the rectal pads were measured under a compound microscope. Coverslips were supported to minimize distortion and damage of the rectal pads. No stains were required because the ethanol made the rectal pads opaque. The area of each rectal pad was approximated using the equation for the the area of an oval.

Rectal pad number was a species characteristic, but varied between species. There were no consistent differences in rectal pad number between arboreal and terrestrial ants. Both groups contained species with two, three or six rectal

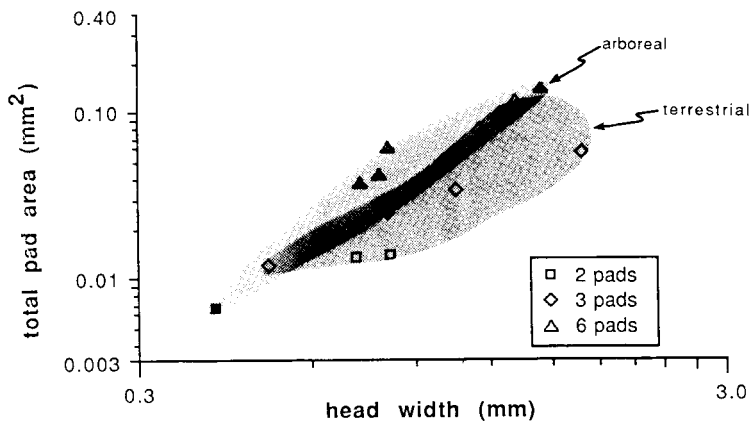


FIG. 3. Total area of rectal pads in a rectum, for ants with two, three or six rectal pads per rectum (log scale). Each point represents the mean of four ants in each of two colonies for each species. Arboreal ant species are represented by solid symbols, terrestrial species by open symbols. Shaded areas include all points representing ants with either two, three or six rectal pads. Dashed lines represent regressions of total pad area (A) on head width (W) for arboreal ($\log A = -1.25 + 2.4 \log W$) and terrestrial ($\log A = -1.48 + 1.93 \log W$) ants, without regard to pad number.

pads. There was, however, a tendency for larger ants to have more rectal pads (Fig. 3). The sample of species was too small to determine taxonomic patterns in rectal pad number (Table 1). However, it is interesting to note that none of the ants in the Poneroid complex have six rectal pads. Even the large *Pogonomyrmex badius* minors have only three pads.

Total rectal pad area increased with body size ($\log[\text{total pad area}] = -1.38 + 2.14[\log[\text{head width}]]$; $R^2 = 74.9\%$, adjusted for degrees of freedom). When the effects of size were removed, total pad area was 1.6 times larger

for arboreal ants than terrestrial (ANCOVA, $F = 4.70$, d.f. = 1, 13, $P < 0.05$; Fig. 3), but average rectal pad area did not differ (ANCOVA, $F = 0.97$, d.f. = 13, $P > 0.20$).

Because surface area is proportional to the square of length for geometrically similar objects, the log transformed relationship between rectal pad area and head width, if isometric, should have a regression coefficient of 2. The common regression coefficient of the comparison of total rectal pad area is 2.14, which is not significantly different from 2 ($t = 1.33$, d.f. = 13, $P > 0.10$).

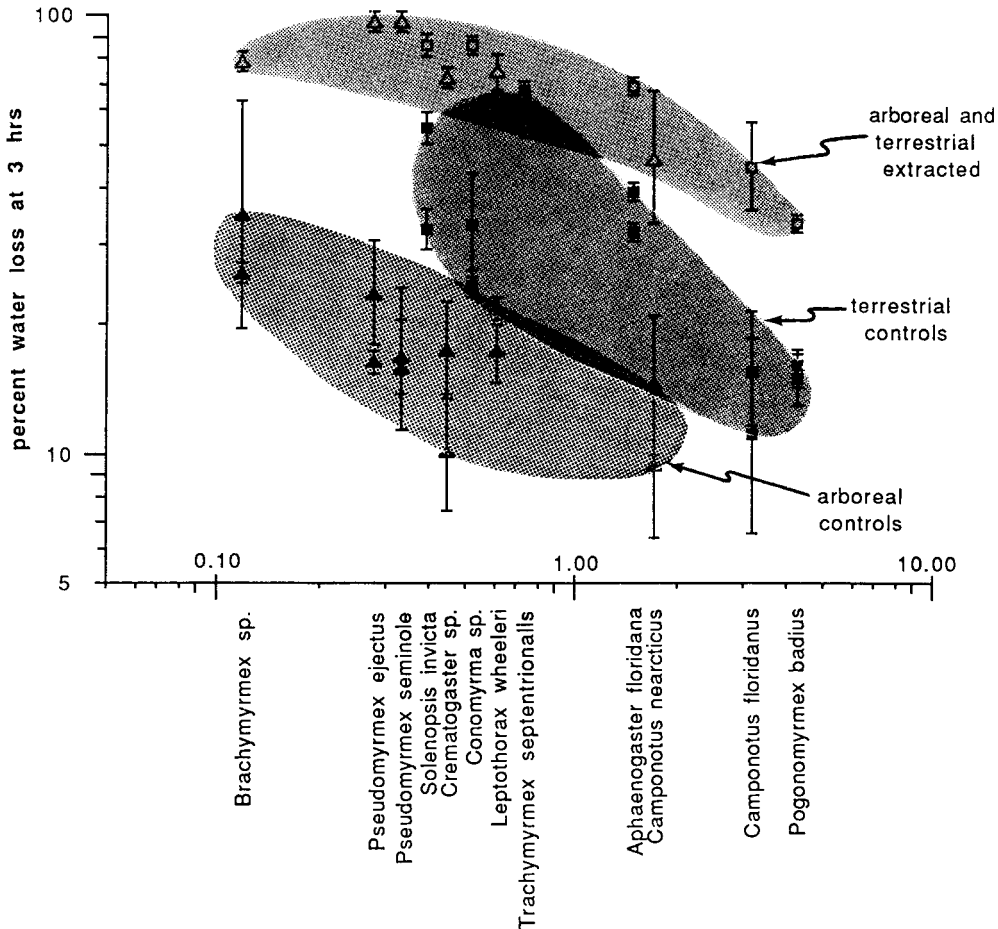


FIG. 4. Percentage water loss for arboreal and terrestrial ant species after 3 h exposure to 0% r.h. Controls (live and dead ants) are represented by closed symbols, dead ants with cuticular lipids extracted by open symbols. Squares indicate terrestrial species, triangles arboreal species. Each point represents the mean of five ants in each of two colonies. Error bars are standard errors. Shaded areas include all points representing either extracted ants, terrestrial controls, or arboreal controls.

Cuticular lipid extractions

Cuticular lipids were removed by bathing freshly freeze-killed ants in a solution of chloroform:methanol (2:1) for various amounts of time (specified later). Ants were weighed before and after extraction. Extracted and unextracted ants were exposed to 0% r.h. at 30°C and their water loss was determined gravimetrically.

A preliminary experiment on similar-sized workers from two colonies each of *S. invicta* and *Crematogaster* sp. was used to determine the optimal extraction and desiccation times for further experiments. Groups of five ants were extracted for 5, 10 or 15 min, or not at all, and then exposed to 0% r.h. for 1, 3, 5 or 7 h. Water loss rate peaked after 15 min of extraction in *Crematogaster* sp., but only 5 min in *S. invicta*. Loss rates for fully extracted ants of both species were similar, but *Crematogaster* sp. extracted 5 and 10 min as well as unextracted lost water more slowly than the corresponding *S. invicta* or the 15 min *Crematogaster* sp. (Tukey's HSD test at $P=0.05$). These results suggest that either *Crematogaster* sp. has more cuticular lipids than *S. invicta*, or the lipids are removed more slowly.

On the basis of this experiment, ants in the follow-up experiment were extracted for 15 min and desiccated for 3 h at 0% r.h. and 30°C to ensure complete removal of cuticular lipids and to allow the greatest resolution of the water loss rates for unextracted ants. The percentage water loss of live, dead and extracted ants of six terrestrial and six arboreal species were compared

(Fig. 4). There were no significant differences in water loss between live and dead ants (controls) for either arboreal or terrestrial species (see also Delyé, 1968). Control arboreal ants lost half as much water as control terrestrial ants. Extraction of both species types resulted in similar water loss, higher than either control, suggesting that cuticular lipids play an important role in the superior desiccation resistance of arboreal ants. Removal of this waterproofing causes extracted ants to lose water at a rate dependent only on their size. Because water loss is proportional to surface area and initial body water content is proportional to volume, percentage water loss for extracted ants should be proportional to A/V or $V^{-1/3}$. Thus, the regression coefficient of the log transformed relationship between percentage water loss and dry weight should be -0.333 . The regression coefficient for the extracted ants was -0.246 , which was not significantly different from the predicted value ($t=1.47$, d.f.=9, $P>0.20$).

Two terrestrial species, *T. septentrionalis* and *A. floridana* (q and a, respectively, in Fig. 1), showed little difference in water loss between extracted and unextracted groups. These species apparently have little or no cuticular waterproofing, perhaps accounting for their unusually low LT_{50} values.

Summary analyses

One should be able to predict LT_{50} s by dividing water loss tolerance by the water loss rates of

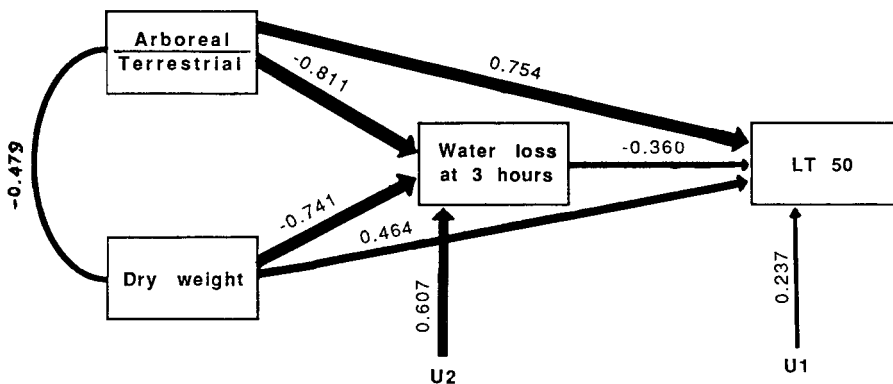


FIG. 5. Path analysis model of the effects of dry weight, water loss rate, and species type (i.e. arboreal or terrestrial) on LT_{50} . Paths, represented by arrows, are drawn from variables taken as causes to variables taken as effects. Paths without arrows represent non-causal correlations. The thickness of the path is proportional to the strength of the correlation that they represent. Correlation coefficients are noted next to each arrow.

live ants ($[\text{water loss at death}(\%)]/[\text{water loss rate}(\%/h)]=\text{hours to death}$). This was done for the species used in the comparisons of water loss tolerance, except *P. pallida* for which data on water loss rate were lacking (Fig. 4). Instead, *P. ejectus* F. Smith, which was not used in the water loss tolerance experiment because it was smaller than other ants in the comparisons groups, was substituted for *P. pallida*. If water loss tolerance and water loss rates fall short in predicting LT_{50} s then some other adaptation to water stress must make a contribution.

The predicted LT_{50} s of terrestrial ants were not significantly lower than their actual LT_{50} s, but the predicted LT_{50} s of arboreal ants were (one-tailed Wilcoxon sign-rank test, $P>0.10$ and $P<0.05$, respectively). Predicted LT_{50} s were an average of 1 h (or 20%) less than actual LT_{50} s for terrestrial ants and 17 h (or 60%) less for arboreal ants. Water loss rates were calculated only for the first 3 h of exposure to 0% r.h. The rate may be non-linear for some arboreal ants. Increasing water loss may induce active reduction of cuticular permeability (Hadley, 1972, 1974) or some other mode of loss reduction.

A path analysis model (Li, 1975) of the effects of dry weight, total rectal pad area, percentage water loss of live ants after 3 h desiccation, and species type on LT_{50} was constructed, based on the ten species in the cuticular extraction experiment for which rectal pad areas were available. The causal effect of total rectal pad area was very small (-5.3%). Using this model as a reference, the adequacy of a second model (Fig. 5), in which the path coefficient between total pad area and LT_{50} was assumed to be zero, was evaluated by a large sample chi-square test (Kim & Kohout, 1975). The two models do not differ significantly ($\chi^2=1.55$, d.f. = 1, $P>0.20$). In the second model species type (arboreal/terrestrial) explained 52.5% of the variation in LT_{50} , water loss rate (% water loss) 31.2%, size (dry weight) 10.7% and 5.6% was unexplained (error). The direct causal effect of species type was more than 2.5 times that of its indirect effects, and the direct effect of size was more than 1.7 times its indirect effects. A model that fully explained the differences in LT_{50} between arboreal and terrestrial ants, while taking into account the effects of size, should result in the direct effects of size and species type being very small, with most of their effects being indirect - acting through other agents, such as water loss rate or

total rectal pad area. Because this is not the case, one must conclude that other factors, not explicitly stated in the model, contribute to the differences in LT_{50} s between arboreal and terrestrial ants.

Water loss rate (%/h) had the expected direct effect on LT_{50} , though it was not of the magnitude suggested by its correlation with LT_{50} . This is because much of its effect results from the indirect effects of species type and dry weight. In other words, some ants lose less water because they are arboreal and/or because they are large. Dry weight and species type account for 73% of the variation in percentage water loss, hence the direct effect of water loss, which is that portion which cannot be ascribed to dry weight or species type. This direct effect may reflect differences between species within type.

There was a negative correlation (-0.479) between species type and dry weight which means that, at least in this instance, the arboreal ants were smaller than the terrestrial ants. Although this may be an artefact of the sample used, our impression is that arboreal ants are indeed generally smaller than their terrestrial kindred. For example, the largest of the arboreal ants in this study, *Colobopsis impressa* (Roger) (once classified as a *Camponotus*) and *Camponotus nearcticus* Emery, are notably smaller than their terrestrial cousins. Although selection may favour large size in arboreal ants to reduce the surface area to volume ratio, space is probably at a premium for arboreal ants, so counterbalancing selective pressures may keep arboreal ants small.

Discussion

In the pine forests of North Florida during summer, water is apparently scarce and heat very abundant. It seems an inhospitable place, and yet only 10 or 15 cm below the top layer of hot, dry sand lies a surprisingly cool, moist reprieve from the relentless heat. Terrestrial ants can take advantage of this reprieve; arboreal ants, nesting in bone-dry, dead weed stems or tree bark, cannot. Because they cannot avoid desiccation stress, arboreal ants must confront it. To this end they have evolved more effective epicuticular lipid waterproofing and other means of retarding water loss. Terrestrial ants are less effectively protected.

Negative correlations between water loss rates and the quantity of epicuticular lipids have been found in many arthropods, including adults and juveniles of the same species (Arnold & Regnier, 1975; Hadley, 1980; Hadley & Warburg, 1986). Thus, it is likely that quantitative differences in cuticular lipids play a major role in the differences in water loss rates between unextracted arboreal and terrestrial ants. This is suggested indirectly by comparisons of *S. invicta* and *Crematogaster* sp. which were not extracted or had been extracted for 5, 10 and 15 min. Alternately, perhaps lipids are more tightly bound and thus more slowly extracted from the epicuticle of *Crematogaster* sp., but because binding does not necessarily lead to greater waterproofing, a simpler explanation is that a greater quantity of lipids causes greater waterproofing and requires more time to be extracted.

Spiracular responses to low humidity reduce the transpiration of water from the tracheae of some arthropods (Krafsur, 1971; Hadley, 1974). However, it is unlikely that there are differences in spiracular behaviour between arboreal and terrestrial ants. Neither we nor Delyé (1968) found differences in the water loss rates of live and dead ants, and spiracular status after death seems an unlikely cause of the differences in water loss rate between dead arboreal and dead terrestrial ants. There is no reason to suppose that the state of the spiracular valves in dead ants should differ between the two species types. Delyé (1968) found that below 35°C the spiracles of live ants are generally closed and that above 35°C they are open. He suggested that this was a method of evaporative cooling, though it may simply be a response to increased oxygen demand at higher temperatures. Because our experiments were conducted at 30°C, it is likely that the spiracles of both species types were closed.

The differences in total rectal pad area between terrestrial and arboreal ants suggest that there may be corresponding differences in the amount of water reclaimed from the faeces. However, the path analysis indicates that differences in rectal pad area have little causal relation to differences in LT_{50} . The ants in this study were not fed during their ordeal. Without feeding and consequent defaecation the gut is not a source of water loss or gain. Under chronic exposure to less severe conditions where feeding is

allowed, the differences in total rectal pad area may contribute significantly to the water balance of arboreal ants. There could also be qualitative differences in rectal pads, which would further enhance the ability of arboreal ants to reclaim faecal water. Detection of these differences would require the use of physiological experiments, together with histology, or electron microscopical examination.

The production of metabolic water by arboreal ants could contribute to their longevity under dry conditions. However, evidence of metabolic water production in response to low humidity has been shown for only one ant species, *Pogonomyrmex rugosus* Emery (Ettershank & Whitford, 1973). Six other desert species had either no response to humidity or responses correlated with increased activity as well as increased mortality (Ettershank & Whitford, 1973; Kay & Whitford, 1975). In addition, comparisons of observed and predicted LT_{50} s used the variable 'percentage water loss till death', to calculate predicted LT_{50} s. Any metabolic water would have been included in this term, which together with the water loss rate, was insufficient to predict LT_{50} s for arboreal species. Arboreal ants weighed an average of 0.99 mg (0.33 mg dry wt) and had LT_{50} s of about 28 h. Their initial water loss rate (0.04 mg/h) predicted an LT_{50} of 11 h. If the 17 h gap could be bridged by metabolizing fat to produce water (the most generous scenario) these ants would need to have metabolized 0.79 mg of fat prior to their death, more than their total dry weight. Thus, it is doubtful that metabolic water production contributes to the high LT_{50} s of arboreal ants.

Arthropods adapted to arid environments can withstand losses of 40–67% of their normal water content (Arlian & Wharton, 1974; Arlian, 1979; Toolson & Hadley, 1977). The water loss tolerances of arboreal and terrestrial ants (51% and 43% respectively) are well within this range and comparable to those of Saharan ants (48%; Delyé, 1968).

After the effects of size are removed, differences in desiccation resistance still exist between species within each species type. For some species these differences are reflected in their patterns of daily activity. Studies showing a correlation between foraging activity and temperature are relatively common, but relatively few studies have investigated correlation between humidity and foraging activity. Field studies can-

not easily separate the effects of r.h. and temperature because they are correlated. Despite their obvious limitations in the study of foraging behaviour, laboratory experiments allow temperature and r.h. to be individually manipulated. Whitford *et al.* (1975), in one of the few studies comparing foraging activity to desiccation resistance, found no relationship between the desiccation resistance and the foraging ecology of the six desert ant species in their study. However, such a relationship does exist among some of the terrestrial ants of our study.

For terrestrial ants of their size, *T.septentrionalis* and *A.floridana* are especially vulnerable to low humidity. They are also the first to retire on a hot day. After 09.00 or 10.00 hours they have retreated to at least 10–15 cm below the surface (unpublished observations). *P.imparis*, which is similarly vulnerable to low humidity, rarely encounters desiccating conditions because it is a winter-active ant (Tschinkel, 1988b). Indeed, Talbot (1943) noticed that high humidity favoured the activity of *P.imparis* and that they were always unusually active after a rain. *P.phantasma*, a species having low to intermediate LT_{50s} , is active yearlong and is also unlikely to encounter desiccation stress because it forages only at night (Trager, 1984).

Potts *et al.* (1984) attempted to correlate the humidity preferences of four species of fire ants (including *S.geminata* and *S.invicta*) with their geographic distributions. They found no differences in preferences. However, our study showed that *S.invicta* has LT_{50s} approximately 1.3 times those of *S.geminata*. In North Florida, *S.invicta* is limited to the margins of seasonal ponds and heavily disturbed, sunnier areas, as well as areas with a high water table. *S.geminata* is found wherever *S.invicta* is not, in less disturbed, shadier areas where the water table is deeper (Tschinkel, 1988a). Our LT_{50} data suggest that the greater resistance of *S.invicta* to desiccation allows its workers to forage in areas of high insolation almost 3 h longer than workers of *S.geminata*, perhaps contributing to their relative distributions. Furthermore, the three terrestrial species having the highest LT_{50s} after the effects of size were removed, *Conomyrma* sp., *S.invicta* and *P.badius*, all nest and forage in areas with high insolation.

Delyé (1968) compared the water loss rates of dead ants from the Sahara and Marseille at 30°C and 0% r.h. Whitford *et al.* (1975) reported the

water loss rates of six live Chihuahuan ant species (rates presumably similar to those of dead ants) and claimed that they were similar to the water loss rates of Delyé's Saharan ants. Neither study used ANCOVA in their comparisons. ANCOVA and *a posteriori* pairwise comparisons show that when the effect of size is removed there are no differences in water loss rates between the terrestrial ants of our study and those from Marseille, nor between arboreal and Saharan ants. However, arboreal and Saharan ants have lower water loss rates than terrestrial ants from Tallahassee and Marseille. The water loss rates of the Chihuahuan ants are intermediate to those of the other ants and do not differ significantly from any of them. That the water loss rates of Chihuahuan desert ants differ only slightly from arboreal ants while their LT_{50s} are significantly lower gives further support to the idea that cuticular waterproofing is only one of perhaps several adaptations to chronic humidity stress. Therefore, comparisons of water loss rates between arboreal, Saharan and Chihuahuan ants may not necessarily correspond with comparisons of the LT_{50s} .

It is our impression, as well as Delyé's (1968), that at depths of 50 cm (sometimes less) the r.h. within the nests of ground-dwelling ants approaches saturation. The r.h. of the nests of arboreal ants probably approaches saturation only during rainfall. The low LT_{50s} of Chihuahuan desert and Floridian terrestrial ants and the high LT_{50s} of arboreal ants suggest that nest r.h. has strongly influenced the development of desiccation stress adaptations in ants. Foraging environment has had only a secondary influence. A comparison of the LT_{50s} of Saharan ants, rather than water loss rates, would serve to further test this hypothesis. We would expect Saharan ants to have LT_{50s} more similar to those of other terrestrial ants than to those of arboreal ants.

Acknowledgments

We are grateful to Dr Duane Meeter for statistical advice and to Dr James C. Trager for species identifications. This work was supported in part by a National Science Foundation Graduate Fellowship and National Science Foundation Grant No. BSR 8502969.

References

- Arlan, L.G. (1979) Significance of passive sorption of atmospheric water vapor and feeding in water balance of the rice weevil, *Sitophilus oryzae*. *Comparative Biochemistry and Physiology*, **67A**, 725–733.
- Arlan, L.G. & Veselica, M.M. (1979) Water balance in insects and mites. *Comparative Biochemistry and Physiology*, **64A**, 191–200.
- Arlan, L.G. & Wharton, G.W. (1974) Kinetics of active and passive components of water exchange between the air and a mite, *Dermatophagoides farinae*. *Journal of Insect Physiology*, **20**, 1063–1077.
- Arnold, M.T. & Regnier, F.E. (1975) A developmental study of the cuticular hydrocarbons of *Sarcophaga bullata*. *Journal of Insect Physiology*, **21**, 1827–1833.
- Bryant, J.L. & Paulson, A.S. (1976) An extension of Tukey's method of multiple comparisons to experimental designs with random concomitant variables. *Biometrika*, **63**, 631–638.
- Coenen-Stass, D. (1986). Investigations on the water balance in the red wood ant *Formica polyctena*: workers, their larvae, and pupae. *Comparative Biochemistry and Physiology*, **83A**, 141–147.
- Delyé, G. (1968) Recherches sur l'écologie, la physiologie et l'éthologie des Fourmis du Sahara. Theses présentées a la Faculté des Sciences de l'Université d'Aix-Marseille.
- Ettershank, G. & Whitford, W.G. (1973) O₂ consumption of two species of *Pogonomyrmex* harvester ants. *Comparative Biochemistry and Physiology*, **46A**, 605–611.
- Gösswald, K. (1938) Über den Einfluss von verschiedener Temperatur und Luftfeuchtigkeit auf die Lebensäusserungen der Ameisen. *Zeitschrift für Wissenschaftliche Zoologie*, **151**, 337–381.
- Hadley, N.F. (1972) Desert species and adaptation. *American Scientist*, **60**, 338–347.
- Hadley, N.F. (1974) Adaptational biology of desert scorpions. *Journal of Arachnology*, **2**, 11–23.
- Hadley, N.F. (1980) Cuticular lipids of adults and nymphal exuviae of the desert cicada, *Diceroprocta apache*. *Comparative Biochemistry and Physiology*, **65B**, 549–553.
- Hadley, N.F. & Warburg, M.R. (1986) Water loss in three species of xeric-adapted isopods: correlations with cuticular lipids. *Comparative Biochemistry and Physiology*, **85A**, 669–672.
- Kay, C.A. & Whitford, W.G. (1975) Influences of temperature and humidity on O₂ consumption of five Chihuahuan desert ants. *Comparative Biochemistry and Physiology*, **52A**, 281–286.
- Kim, J. & Kohout, F.J. (1975) Special topics in general linear models. In: *SPSS: Statistical Package for the Social Sciences*, 2nd edn (ed. by N. H. Nie, C. H. Hull, J. G. Jenkins, K. Steinbrenner and D. H. Bent). McGraw-Hill Book Co., New York.
- Krafsur, E.S. (1971) Behavior of thoracic spiracles of *Aedes* mosquitoes in controlled relative humidities. *Annals of the Entomological Society of America*, **64**, 93–97.
- Li, C.C. (1975) *Path Analysis: A Primer*. Boxwood Press, Pacific Grove, California.
- Machin, J., O'Donnell, M.J. & Coutchié, P.A. (1982) Mechanisms of water vapor absorption in insects. *Journal of Experimental Zoology*, **222**, 309–320.
- Potts, L.R., Francke, O.F. & Cokendolpher, J.C. (1984) Humidity preferences of four species of fire ants. *Insectes Sociaux*, **31**, 335–339.
- Sigal, M.D. & Arlan, L.G. (1982) Water balance of the social insect *Formica exsectoides* and its ecological implications. *Physiological Zoology*, **55**, 355–366.
- Talbot, M. (1943) Response of the ant *Prenolepis imparis* (Say) to temperature and humidity changes. *Ecology*, **24**, 346–352.
- Toolson, E.C. & Hadley, N.F. (1977) Cuticular permeability and epicuticular lipid composition in two Arizona Vejovid scorpions. *Physiological Zoology*, **50**, 323–330.
- Trager, J.C. (1984) A revision of the genus *Paratrechina* (Hymenoptera: Formicidae) of the continental United States. *Sociobiology*, **9**, 51–162.
- Tschinkel, W.R. (1988a) Distribution of the fire ants *Solenopsis invicta* and *S. geminata* in North Florida in relation to habitat and disturbance. *Annals of the Entomological Society of America*, **86**, 76–81.
- Tschinkel, W.R. (1988b) Seasonal life history and nest architecture of a winter-active ant, *Prenolepis imparis*. *Insectes Sociaux*, **34**, 143–164.
- Whitford, W.G., Kay, C.A. & Schumacher, A.M. (1975) Water loss in Chihuahuan desert ants. *Physiological Zoology*, **48**, 390–397.
- Wilson, E.O. (1971) *The Insect Societies*. Harvard University Press, Cambridge, Mass.