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UNIVERSITY OF CALIFORNIA
RIVERSIDE

Food Preference, Survivorship, and Intraspecific Interactions of Velvety Tree Ants

A Thesis submitted in partial satisfaction of the requirements for the degree of

Master of Science

in

Entomology

by

Rochelle Viola Hoey-Chamberlain

December 2012

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ABSTRACT OF THE THESIS

Food Preference, Survivorship, and Intraspecific Interactions of Velvety Tree Ants

by

Rochelle Viola Hoey-Chamberlain

Master of Science, Graduate Program in Entomology
University of California, Riverside, December 2012
Dr. Michael K. Rust, Chairperson

*Liometopum apiculatum* Mayr, *L. luctuosum* Wheeler, and *L. occidentale* Emery are found in western North America and are referred to as velvety tree ants. They are usually associated with trees, but in recent years both *L. luctuosum* and *L. occidentale* have been reported as urban pests causing structural damage. Very little is known about the biology of these species.

*Liometopum occidentale* foragers readily consumed sucrose, glucose and honey sucanat solutions. Solid protein baits containing anchovy were retrieved by workers. In the early summer, foragers consumed more of the same types of foods during the day and at night. Even though workers are polymorphic, they all consumed about 0.25 mg of a 25% sucrose solution, providing a mechanism of determining foraging activity by measuring sugar water removal from monitoring stations. Baits containing 25% sucrose would be effective if suitable toxicants can be identified.

*Liometopum luctuosum* is restricted to the coniferous forests in the mountains in the southern range of its distribution, suggesting that it may be less xeric adapted than *L.*
occidentale. When exposed the various temperatures and relative humidities, *L.*
*luctuosum* workers survived significantly longer than *L. occidentale.* Furthermore, *L.*
luctuosum lost significantly more water over 24 h than did *L. occidentale* at 25.6 and
33.0°C. The cuticular permeability (CP) of *L. luctuosum* was 17.8 and 19.1, while CP of
*L. occidentale* was 17.0 and 19.8 at 25.3° and 33°C. The similarity of the CPs and the
comparison of the effect of saturation deficit on survival indicated that water loss alone
through the cuticle was probably not the major factor affecting survival. The CPs of both
species suggests they are adapted for xeric conditions; but, also capable of surviving in
mesic climates.

*Liometopum occidentale* nests are quite inaccessible making it extremely difficult
to examine entire colonies and determine colony structure. A segment of the
mitochondrial (mtDNA) COI gene was used to determine that *L. occidentale* nest contain
either one queen or multiple maternally related queens. Observations of the behaviors
displayed by workers from colonies separated in an urban landscape showed that there is
a slight but significant correlation between geographic distance and their level of
aggression.
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Chapter 1:

The Biology, Ecology and Behavior of Velvety Tree Ants of North America
ABSTRACT

Ants belonging to the genus *Liometopum* are regionally distributed across North America, Europe and Asia. *Liometopum apiculatum* Mayr, *L. luctuosum* Wheeler, and *L. occidentale* Emery are found in western North America and are referred to as velvety tree ants. Most species are associated with trees, but in recent years both *L. luctuosum* and *L. occidentale* have been reported as urban pests causing structural damage. In part this is because these ants are frequently misidentified as carpenter ants and there has been increased urbanization of rural and mountain areas of the western U.S. Very little is known about the biology of these species. The best known species is *L. apiculatum* because it is collected and consumed as “escamoles” by humans in Mexico. The following review is intended to summarize the important literature regarding the biology and control of these species. Special emphasis has been given to factors that might be important in their control and gaps in our current knowledge.

INTRODUCTION

The genus *Liometopum* consists of 9 fossil and 8 extant species scattered over North America, Europe and Asia ([www.antcat.org/catalog/329538](http://www.antcat.org/catalog/329538)). There are three North American species, found in the western U.S. and Mexico: *L. apiculatum* Mayr, *L. luctuosum* Wheeler, and *L. occidentale* Emery. The genus *Liometopum* belongs to the subfamily Dolichoderinae, which also includes other important structural pest ants such as the Argentine ant, *Linepithema humile* (Mayr), and the odorous house ant, *Tapinoma sessile* Say. According to Del Toro et al. (2009), *Liometopum* is only a “minor pest in and
around rural housing areas” and “no severe damages to housing or property have been reported,” although the “uncomfortable bite and smell of this ant makes it a nuisance to affected residents.” However, structural damage has been reported for two species, *L. luctuosum* and *L. occidentale* (Wheeler & Wheeler 1986, Merickel & Clark 1994, Gulmahamad 1995, Hedges 1998, Klotz et al. 2008). Homeowners commonly complain of the odor associated with an infestation of velvety tree ants.

*Liometopum luctuosum* and *L. occidentale* are often mistaken for carpenter ants (*Camponotus* spp.) by homeowners and Pest Management Professionals (PMPs). This mistaken identity is due to morphological and behavioral characteristics they share with carpenter ants; namely polymorphic workers, a smooth convex thoracic profile, and the tendency to excavate wood (Hansen & Kltoz 1999, Klotz et al. 2008). *Liometopum luctuosum* are also often confused with the *T. sessile* since they have the same coloration, are similar in size, and produce an alarm pheromone with a very similar odor. Consequently, their importance as structural pests may be greatly under reported, especially in California, Oregon, and Washington.

All three North American species are easily disturbed and when aggravated, they resort to highly aggressive defensive behaviors, biting and releasing strong alarm odors from their anal glands (Del Toro et al. 2009). They produce a noxious alarm pheromone (described as smelling like rotten coconut or having an intense butyric acid-like odor). This pheromone consists of 6-methyl-5-hepten-2-one, acetic acid, n-butyric acid, and 3-methylbutyric acid (isovaleric acid) (Casnati et al. 1964). Alarm pheromones of many dolichoderines seem to be less species-specific than olfactory sex attractants and other
pheromones. For instance, the alarm pheromones of *Forelius pruinosus* (Roger), *Dolichoderus bispinosa* (Olivier), *L. occidentale*, and *T. sessile* will mutually affect one another. In *L. occidentale* this pheromone originates in the anal gland (Wilson & Pavan 1959).

In Mexico, colonies of *Liometopum* have been used as a food resource by people in rural areas for centuries. The immature stages of the reproductive caste, known as “escamoles” are consumed and are a high-quality source of protein, carbohydrates, and lipids. Adult reproductives may also be consumed by humans during swarming, and worker brood is consumed when other stages are scarce (Ramos-Elorduy & Levieux 1992). Consequently, considerably more is known about the biology of *L. apiculatum* than any of the other species.

**HABITAT/DISTRIBUTION**

*Liometopum apiculatum* ants are found in arid and semi-arid regions of southwestern U.S. and Mexico to Quintana Roo (Del Toro et al. 2009). They extend from Colorado through Texas, New Mexico, and southeastern Arizona, and south into Mexico (Gulmahamad 1995). They are usually found at elevations between 1000 and 2500 m, but their prime habitat is oak forests found around 2000 m. At higher elevations they are found in pinyon pine zones, up to the ponderosa pine and riparian zones; at lower elevations they inhabit creosote bush scrub and grasslands in microhabitats of clay, under rocks, boulders, and decaying logs (Del Toro et al. 2009). They have also been found in foothill meadows, deciduous canyon forests, pinyon-cedar woodlands, ponderosa pine-
cedar-oak woodlands, and cottonwood-willow forests (Mackay & Mackay 2002). At high elevations, their abundance decreases and they are replaced by *L. luctuosum* (Del Toro et al. 2009). Altitude may play an important role in the distribution of these ants. In regions of Mexico explored by Conconi et al. (1983b), *Liometopum* are only found between 2000 and 3000 m. Although conditions below 1800 m looked favorable, they are absent. In the U.S., *L. apiculatum* is found from 1316 to 2438 m.

*Liometopum luctuosum* has been reported at elevations as low as 59 m (Dr. Laurel Hansen, Spokane Community College, personal communication, Sept. 6, 2012), but is typically found at elevations higher than 2400 m in more southern latitudes (Wheeler & Wheeler 1986, Del Toro et al. 2009). The range of elevation of this species also appears to depend on latitude with elevations as low as 59 m from collections in Washington state up to 1596 m from collections in California (WA: 59-724m, ID: 664-786m, OR: 277-454m, CA: 1280-1596m, and NV: 1372-2469m; Dr. Laurel Hansen personal communication, Sept. 6, 2012, personal collection) and above 2000 m in New Mexico (Mackay & MacKay 2002). Their range extends from temperate habitats as far north as British Columbia, and to more arid habitats of Central Mexico and western Texas. They inhabit pine, oak, Douglas fir, and juniper forests, sagebrush, and high-elevation riparian habitats (Conconi et al. 1987a, Clark & Blom 2007). This species is often strongly associated with but not limited to pine trees (Del Toro et al. 2009).

*L. occidentale* is found from sea level to over 1700 m in coastal regions from southern Washington to northern Mexico (Snelling & George 1979, Del Toro et al. 2009, Dr. Laurel Hansen, personal communication, Sept. 6, 2012). The range of elevation of
this species also appears to depend on latitude with ants collected from locations in Oregon as low as 7 m and up to 1700 m in California (WA: 31-142m, OR: 7-348m, CA: 142-1713m; Dr. Laurel Hansen, personal communication, Sept. 6, 2012, personal collection). They are the most common and dominant ant in oak and pine forests of southwestern U.S. (Wheeler & Wheeler 1986, Ward 2005, Del Toro et al. 2009). They prefer to nest in the crevices of oaks, alders, elms, cottonwoods, and creosote, and in soil, underneath bark of dead trees and under rotten logs (Cook 1953).

Within their distribution range, the elevations at which Liometopum are found decreases the further north the location. To definitively demonstrate that there is an effect of latitude on the elevation at which Liometopum is distributed in North America, a larger collection of these ants must be made.

NESTS

Nests of L. apiculatum are typically located underground and have a very distinctive structure. They fill hollowed-out chambers with a branched network of carton-like material made out of agglomerated soil and oral secretions until the entire nest resembles Swiss cheese. Within the nest as many as 3 or 4 large chambers containing this honeycombed carton-like material can be found. The carton-like material of this species is much finer than that of L. occidentale. L. apiculatum tend to nest at higher densities than do L. occidentale (Conconi et al. 1987a). These ants are usually found nesting in dead logs, under stones, or in decaying stalks of Yucca spp. (Miller 2007). They have also been collected in glass containers and rubber tires (Del Toro et al. 2009) and among the
roots of various perennial xerophytes such as Agave spp. L., Opuntia spp. Mill., Myrtillocactus geometrizans Console, Yucca filifera Chabaud, Senecio praecox DC., Schinus molle L. or Prosopis juliflora DC. (Conconi et al. 1983b). In some habitats, the nests are deep under heavy boulders or large trees (Wheeler 1905, Gregg 1963a). The queen is always well protected and is usually in a remote place about 6-8 m from the largest chamber where the brood is stored. The chambers are connected by various galleries.

**Liometopum luctuosum** nest under rocks, decaying logs or at the base of large trees, or among the roots of trees such as Quercus spp., Juniperus spp. and Pinus spp. (Conconi et al. 1983b). They create carton nests similar in structure to *L. apiculatum* (Del Toro et al. 2009). Similarly, the queen of *L. luctuosum* is well protected at a remote place about 6-8 m from the largest chamber where the brood is stored, and the chambers of the nest are connected by various galleries (Conconi et al. 1987a).

**Liometopum occidentale** typically nest in soil, crevices of trees, and under the bark of dead trees (Del Toro et al. 2009). Furthermore, nests containing brood and queens are difficult to locate which lead Wang et al. (2010) to speculate that they may also nest deep under large boulders or among roots of large trees. To better understand the structure of their nests, more nests need to be excavated.

**COLONIES**

Colonies of *L. apiculatum* are polydomous with segments of nests (or satellite colonies) scattered over the landscape (Del Toro et al. 2009). Colonies are variable in
size, ranging from a few hundred to hundreds of thousands of workers (Gregg 1963b, Ramos-Elorduy & Levieux 1992). Colonies exploited for their brood by humans contain between 65,000 and 85,000 individuals, while undisturbed colonies may contain as many as 250,000 individuals. Colonies remain useful for repeated brood collection by humans for 4 to 12 years (Ramos-Elorduy & Levieux 1992).

Colony foundation in *L. apiculatum* is by haplometrosis (non-cooperative), that is, a single fertile queen founds each colony (Conconi et al. 1987a,b). Colony foundation behavior is not uniform among founding queens. The time spent exploring, excavating, and removing excavated materials and waste are usually higher throughout the day, while oviposition, brood care, and inactivity increase at night (Conconi et al. 1987b). Founding queens prefer sites close to bodies of water; however, sites slightly further from water are more conducive to the establishment of a successful colony as persistently high humidity will result in the early death of a colony due to fungal invasion (Conconi et al. 1983a).

Colony foundation in *L. luctuosum* is by pleometrosis (cooperative), in which 2 to 40 fertile queens found a single colony (Conconi et al. 1987a). Most colony foundation activities take place at night (Conconi et al. 1987b). There is a division of labor among founder queens. However, this is not always divided the same for each foundation event. The amount of time dedicated to each activity by each queen varies with each colony foundation event. Some queens are active in a variety of tasks. For example, some queens dedicate more time to brood care and others more time patrolling the nest area. The fewer ants founding together (3 or less), the more time spent per individual caring for brood, ovipositing, and exploring. Trophallaxis, patrol activity, and inactivity decreases in these
cases, but brood care still remains the primary activity of founding queens (Conconi et al. 1987b).

After finding no aggression between workers collected from significant distances apart and no territorial boundary, Wang et al. (2010) speculated that *L. occidentale* colonies are large and polydomous. Since they never found brood or queens, it is uncertain whether there are multiple queens within a nest, or whether each queen has some localized “sphere of exclusivity.” and it just seems unlikely that there could be just one queen that produces enough eggs to establish a colony that is one kilometer wide, they also speculated that *L. occidentale* are polygyne. Colonies have been estimated to contain between 40,000 and 60,000 workers (Ramos-Elorduy & Levieux 1992, Del Toro et al. 2009). Colony foundation of this species has not been studied as well as it has been with the other two North American species.

**FEEDING**

*Liometopum apiculatum* are opportunistic carnivores and granivores, and have been observed foraging on dead insects, larger colonies being more predaceous (Shapley 1920). *Liometopum apiculatum* also feeds on crustaceans, annelids, mollusks, dead vertebrates, animal droppings, and extrafloral nectar (Velasco et al. 2007). These ants also obtain nectar or pollen from bear grass and substances from the outside of the ovaries of the flowers of century plants (*Agave scabra* Salm-Dyck and *A. chisosensis* C.H.Mull.) and Spanish dagger (*Yucca* spp.). Workers have been attracted to various foods used as baits including apple sauce, sausage, vegetable soup, sugar water, and
cookies. *Liometopum apiculatum* have also been observed soliciting honeydew from insects including membracids (*Vanduzea segmentata* [Fowler]), aphids, and other ants (*Pogonomyrmex barbatus* (Smith, F.), *Camponotus sayi* Emery, and *Solenopsis xyloni* McCook (Van Pelt 1971). In some habitats the honeydew produced by hemipterans, *Cinara* spp., *Dysmicoccus brevipes* (Cockerell) and *Saissetia oleae* (Oliver), are the main energy sources (Velasco et al. 2007). In other words, hemipteran exudates make up the bulk of the diet of *L. apiculatum* (Conconi et al. 1983b). Their role in disrupting biological control has not been determined.

*Liometropum occidentale* are opportunistic omnivores (Wheeler 1905) and can often be found tending hemipterans and carrying prey insects back to the nest (Conconi et al. 1987a, Gulmahamad 1995). They readily attend hemipterans and are found in citrus groves, but their role in disrupting biological control has not been determined. Their feeding preferences need to be studied to enable the development of effective baits for pest control purposes.

The feeding preferences and habits of *L. luctuosum* have not been reported. This aspect of their biology should be studied to assist in bait development for pest control in structures.

**FORAGING ACTIVITY**

Ants of the genus *Liometopum* lay down a trail pheromone that has an odor similar to butyric acid, but its chemical composition remains unknown. The source of trail pheromones in many species of Dolichoderines is the Pavan gland, a medioventral
sac between the sixth and seventh abdominal sternites (Pavan 1955, Billen 1985). This gland may be the source of these pheromones in *Liometopum*. All the dolichoderine trail pheromones tested so far have proved to be highly species-specific (Wilson & Pavan 1959).

Dolichoderine foragers travel along trials in a manner described by Shapely (1920) as “trail-running,” which I have observed as travelling rapidly along defined trails. The number of ants on these trails is governed largely by food and the speed of the ants by meteorological conditions. Shapley (1920) noted that *Liometopum* activity patterns are equally diurnal and nocturnal. *Liometopum apiculatum* and *L. occidentale* are active at wide range of temperatures (8°C - 38°C), humidity (5%-100%), wind, and light, but temperature is the most important factor affecting their activity. Even in winter after a few warm days these ants have been observed foraging within a few feet of snow banks (Shapley 1920), an example of how imperative temperature is to foraging activity of these ants. *Liometopum* appear to run at a speed very near the maximum speed possible under prevailing conditions, except at low temperatures. For temperatures below 15°C, in which the activity level of these ants is very low, activity can be temporarily increased by exciting the ants into battle or by the discovery of food (Shapley 1920).

Foraging trails for *L. apiculatum* and *L. occidentale* are maintained over long intervals of time and even years (Shapley 1920). Ants on these trails are for the most part unburdened with prey or objects, no matter if they are going toward or away from the nest. Shapley (1920) suggests they are just patrolling, however, what is equally as likely
is that these workers are carrying large amounts of honeydew or other liquid foods within their crop.

*Liometopum apiculatum* forage from March to September (Mackay & Mackay 2002). Workers forage almost exclusively on trails as wide as 2-3 cm on the soil surface, and when the temperature rises sharply at midday, they cease foraging and seek shelter under stones (Conconi et al. 1983b, Ramos-Elorduy & Levieux 1992). The movement of this species is less erratic than *L. occidentale* at higher temperatures. An increase in temperature by 30°C changes the speed by 15 fold, increasing exponentially from 0.44 to 6.60 cm a second. There also appears to be little difference in the speed whether ants are moving towards or away from the nest, or between large and small workers during the summer months. However, after prolonged periods (two months or more) of low temperatures, the larger workers are faster than the small workers. Within a range of 14º to 38ºC, there appears to be little effect of temperature on the number of ants on trails. Maximal activity occurs between 12p.m. - 12a.m. during the summer months in southern alpine habitats such as Mount Wilson, CA (Shapley 1920). In natural environments, ants of this species forage in areas between 468 and 708 m² (= 612 m²); however, they only use between 16 to 30% of this area at any given time. The spatial distribution of the foraging areas for these species seems to be strongly correlated with the location of shrubs and trees infested by hemipterans (Ramos-Elorduy & Levieux 1992).

In natural environments, *L. occidentale* forages an area as large as 2,000 m², but they only utilize between 486 and 1198 m² (= 740 m²) of this area at any one time. This
means that they are only using between 33 and 68% of this area, more than twice that of
According to Ramos-Elorduy and Levieux (1992), *L. occidentale* travels mostly
underground in very shallow galleries (1-2 cm deep), or in the litter. However, I have
seen very long trails above ground. These ants form massive foraging trails over 60 m in
length with some trails as long as 145 and 185 m (Conconi et al. 1983a,b; Gulmahamad
1995), and can even be observed on hot days with temperatures between 24 and 38°C
(Tremper 1971). Both *L. luctuosum* and *L. occidentale* can be seen foraging during the
day in great numbers in the spring and early summer, but around mid-summer they
switch to night time foraging. However, this has not been scientifically proven.

**INTERSPECIFIC INTERACTIONS AND NESTMATE RECOGNITION**

*Liometopum* are highly competitive, behaviorally dominant ants and play an
ecologically similar role to the behaviorally dominant Australian dolichoderines,
*Anonychomyrma, Papyrius* and Froggattella (Andersen 1997). Ant communities often
have a hierarchal order to them, with “submissive” ant species being more adaptable, and
“territorial” species at the top of this hierarchy defending territories of varying sizes
(Petráková & Schlaghamerský 2011). Ants fight in the spring when food is scarce and
also fight to renew trails after winter pauses in activity (Petráková & Schlaghamerský
2011). The interactions between *Liometopum* spp. and native ants have been studied for
*L. microcephalum* Panzer, an Old World species. *L. microcephalum* is a behaviorally
dominant ant species in Europe and Asia. This species builds nests several meters above
ground in old living trees (especially oaks) and forage in other trees in the vicinity of the nest tree, similar to North American species of *Liometopum* (Petráková and Schlaghamerský 2011). Some colonies are polydomous. These ants are very efficient hunters, but also tend aphids. *Liometopum microcephalum* can be very aggressive toward other ant species, attacking by biting and spraying secretions that repels enemies, and initiating alarm behavior (Petráková & Schlaghamerský 2011). Aggressive behavior occurs close to the nest, on trails, on trees, and occasionally at food resources.

*Liometopum microcephalum* takes advantage of worker cooperation during aggressive interactions, a strategy used by smaller ants (Petráková & Schlaghamerský 2011). Aggressive intraspecific interactions might be expected with all three North American species due to such similarity in ecology and behavior of this species.

In Tlaxco, Tlaxcala, Mexico, *L. apiculatum* was found to associate with fourteen species of Hemiptera, including seven species of aphids (two in the *Cinara* genus, *Anoecia cornicola* (Walsh), *Aphis lugentis* Williams, *Aphis solitaria* (McVicar Baker), *Aphis helianthi* Monell in Riley & Monell, and *Aphis* spp.), three species of scales (*Saissetia* genus, *S. olee* (Olivier), two species of *Pseudococcidae* including *D. brevipes* [Cockerell]); and one species of *Ortheziidae*, and one species of *Dactylopiidae* (*Eriococcus* spp.) (Velasco et al. 2007). Yet they associate with another species of hemipteran, the cochineal scales *Crassicoccus* sp. that live on oak trees.

Competition between the invasive *L. humile* and *L. occidentale* has been documented (Ward 1987, Holway, 1998, Sanders et al. 2003). However, *L. occidentale* rarely co-exists with *L. humile*, perhaps because they are habitat or resource specialists.
that are found in such a wide range of habitats as *L. humile* or because they are weak competitors against invasive species (Sanders et al. 2003). However, *L. occidentale* is the species of *Liometopum* most affected by *L. humile* due to its “taxonomic and ecological similarities to *Linepithema humile* in that “they are members of the same subfamily (Dolichoderinae) and they are dominant, opportunistic, epigaeic (lives or forages primarily above ground) ants, with propensities to establish dense foraging trails, to tend *Hemiptera: Sternorrhyncha*, to move nest sites readily, and to forage and tend hemipterans under the same ambient conditions throughout the summer months (Ward 1987). ” This abundance of similarities makes them likely to compete in areas of co-occurrence.

Wang et al. (2010) tested the nest mate recognition of a limited number of *L. occidentale* colonies in James Reserve and Stunt Rance in southern California. Results of this study showed that ants from sites separated by more than a kilometer were not aggressive to one another, while colonies separated by more than 100 km were aggressive to each other; however, some of these colonies were not aggressive to each other. Aggression was not uniform between all pairings, meaning that some colonies are more aggressive than others. Wang et al. (2010) also tested the aggression of *L. occidentale* toward other ant species, *Camponotus vicinus* Mayr, *Myrmecocystus ewarti* Snelling, *M. testaceus* Snelling, *Pogonomyrmex subnitidus* Emery, *Solenopsis maniosa* McCook and *Tapinoma sessile* Say, and found that they were highly aggressive to these species.

*Liometopum luctuosum* “has been reported as being a competitor of *Camponotus* species in Idaho, since both of them compete for similar nesting sites” (Merickel & Clark
1994). However, *L. luctuosum* is the least studied of all the North American *Liometopum* species.

**POLYMORPHISM**

Polymorphism in *L. apiculatum* has been described as bimodal with diphasic allometry (Conconi et al. 1987a). Bimodal or biphasic allometry occurs when animals such as ants have structures that vary disproportionately with body size such as the heads and mandibles of soldiers and major and minor workers (Grimaldi & Engel 2005). At some intermediate, boundary size, larger individuals have a disproportionately larger structure, but the structure is disproportionately small below this size (Grimaldi & Engel 2005). The average live weight of the largest workers of *L. apiculatum* is about 3.24 mg and the average weight of the minor workers are half this, but the extremes of weight for majors and minors are probably in the ratio of four to one (Shapely 1920). The polymorphism of *L. occidentale* and *L. luctuosum* has not been described.

**REPRODUCTION**

Immature stages of reproductives have been found in *L. apiculatum* nests from May to August, whereas the rest of the year the brood is of the worker caste (Conconi et al. 1983a, Del Toro et al. 2009). Males and gynes have been collected outside the nest from June to August and queens (likely founding queens) have been collected in July and August under stones and other landscape features (Del Toro et al. 2009). Nuptial flights of this species occur during the day after a heavy rain during the months of April or May.
(Conconi et al. 1983a). Before a nuptial flight there is a great agitation of the workers, which leave the nest and run rapidly in a “zig-zag” fashion. The male and female alates leave the nest, but are less active. After a while the workers begin to bite the legs and wings of the alates, forcing them to climb the nearest plant. The workers continue to excite the alates with bites until they begin to beat their wings, and subsequently initiate flight one by one, not as a swarm. Mating takes place in the air, and mated males and females fall to earth together, often still attached (Conconi et al. 1983a). The life span of *L. apiculatum* queens is shorter than that of *L. luctuosum* queens (exact time difference not specified) but, their productivity (oviposition) is greater (Conconi et al. 1987a).

The annual productivity for an established colony (60 to 85,000 workers) of *L. apiculatum* is about 3-3.6 kg of brood per year (Ramos-Elorduy & Levieux 1992). Oviposition by founding queens is large, but only a small percentage reaches the adult stage of the F1 generation, partly because the smaller, “trophic” eggs are consumed as food. After laying her first batch of eggs, the queen delays laying more until the first eggs have developed into pupae. Once the first workers emerge, the queen discontinues laying trophic eggs, which lowers the total amount of eggs laid but increases the proportion of viable eggs. Eggs are laid all year round (Conconi et al. 1983a).

Some virgin queens of *L. apiculatum* emerge from the nest, remove their wings, and dig a nest without mating. They will lay eggs, care for them, and eat them to survive. However, they only care for more recently laid eggs that have not turned yellow or dried out (Conconi et al. 1983a).
Flights of *L. occidentale* reproductives have been observed throughout May (Del Toro et al. 2009). The annual productivity for a colony (40 to 60,000 workers) of this species is 2 to 2.8 kg of brood per year for 4-8 years (Ramos-Elorduy & Levieux 1992). Workers housed without a queen will also lay unfertilized eggs that are eaten or develop into males.

Reproductives of *L. luctuosum* have been observed flying in June and July and can be collected the day after the flight in large bodies of water or using a blacklight trap (Del Toro et al. 2009).

The most productive colonies of *Liometopum* are those that are more substantially surrounded by vegetation which likely contains honeydew excreting hemipterans. *Liometopum apiculatum* appears to be more productive than *L. occidentale* even though *L. apiculatum* forages in a much smaller territory (Ramos-Elorduy & Levieux 1992).

The reproductive life cycles of *L. luctuosum* and *L. occidentale* need further investigation.

**LIFE CYCLE**

A study by Conconi et al. (1983a) recorded the longevity of each of the reproductive castes and colony foundation of *L. apiculatum*. They studied the life cycle of this species under different conditions of humidity, temperature, and substrate. Ant queens were placed either in glass tubes with moist cotton or in jars with soil, and were held at varying temperatures and relative humidity. Observations of the time until different life cycle events occurred are summarized in Table 1.
Observations of the longevity of the various reproductive castes of *L. apiculatum* are as follows: males lived 15 to 37 days, virgin queens lived 19 to 268 days and fertilized queens lived 17 to 316 days. This study is a good start to give us an idea of the duration of different life cycle events and the life spans of each caste; however, further work is needed to understand the variability in time of these events under various conditions.

No such studies have been conducted with *L. occidentale* or *L. luctuosum*.

**ASSOCIATIONS AND MUTUALISMS**

Beetles belonging to the Family Staphylinidae including *Sceptobius schmitti* Wasmann, *Dinardilla liometopi* Wasmann, *Dinardilla mexicana* Mann, and *Sceptobius dispar* Sharp have been found within the nests of *L. apiculatum* (Gregg 1963a, Danoff Burg 1994). “*Dinardilla mexicana* and *S. dispar* currently co-occupy *L. apiculutiim* nests on the eastern part of the Mexican plateau, while *S. schmitti* and *D. liometopi* are found together in nests on the western part of the Mexican plateau and then north to Colorado. More collecting should be done in the central part of the Mexican plateau to determine whether there is a zone of overlap between these two geographical species groups” (Danoff Burg 1994). The staphylinid *Liometoxenus* was first described from specimens found foraging next to colonies of *L. luctuosum* and *L. occidentale* (Kistner et al. 2002, Del Toro et al. 2009). A weevil *Liometophilus manni* Fall has also been discovered in the galleries of *L. apiculatum* in southern Arizona and Mexico (Mann 1914). The impact or role of these beetles on the ants is unknown.
Dinardilla and Sceptobius beetles are also often observed alongside foragers or in nests of L. occidentale. Sceptobius lativentris (Fenyes) is only found with L. occidentale, this is unique among the Sceptobiini (Danoff Burg 1994). Additional collections of L. occidentale nests need to be conducted to determine the current geographic distribution of S. lativentris (Danoff Burg 1994).

All members of the genus Sceptobius are quick moving, long-legged beetles that interact with their ant hosts by running up to groups of ants, briefly grooming a number of them, and then running away to the periphery of the ant nest (Danoff Burg 1994). Dinardilla mexicana and D. liometopi interact actively and, in some cases, aggressively with some host ants. Typically, one of these beetles approaches a stationary host ant from the side and begins grooming the ant’s legs with its mouthparts, after which the beetle mounts the ant and grooms its dorsum. This interaction can last from 3 to 20 minutes and is likely used to spread the cuticular hydrocarbons that specify the colony odor from the ant to the beetle (Danoff Burg 1994).

The staphylinid Liometoxenus was first described from specimens found foraging next to colonies of L. luctuosum and L. occidentale (Kistner et al. 2002; Del Toro et al. 2009).

Liometopum also have mutualistic relationships with both plants and other insects. One example is the mutualistic relationship of L. apiculatum with the cholla cactus Opuntia imbricate (Haw.). This species has been seen protecting the cacti from herbivores and seed predators as well as foraging on extra floral nectars (Miller 2007).
Liometopum apiculatum tends the aphid Cinara spp. 1 found on Pinus rudis Endl. and the aphid Cinara spp. 2 found on Juniperus deppeana Steud. (Velasco et al. 2007).

STRUCTURAL PESTS AND THEIR CONTROL

When Liometopum ants nest inside structures they can produce considerable amounts of frass consisting of chewed wood and insulation (Fig. 1). The excavations of these ants are of a finer texture than those of carpenter ants (Fig. 2) and the frass is also much finer (Fig. 3). Attempts to eradicate nests indoors are often unsuccessful because foragers may congregate in hollows within insulation and wood, forming temporary ‘resting places,’ which are mistaken as nests (Klotz et al. 2008).

Velvety tree ants are best managed by locating and treating all colonies around the structure (Hedges 1998). Inspect for nests and foraging trails in or around wood such as stumps, trees, or landscape timbers (Hansen & Klotz 1999). These ants are attracted to water-damaged wood, but nests can also be found in dry, sound wood and foam insulation. Night inspections might be helpful during summer months as these ants are more active at night (Klotz et al. 2008).

A combination of baits and sprays should be used in an IPM program to successfully manage these ants. Baits containing a sweet food attractant should be applied in or near foraging trails first, allowing time for the ants to carry the bait active ingredient into the nest. Then dusts and sprays may be applied. Dust formulations may be used by PMPs to treat “resting” areas or satellite nests within structures (Hedges 1998, Klotz et al. 2008).
Re-entry by undetected colonies outside a structure can be prevented with a perimeter spray around the foundation of the structure (Hedges 1998, Klotz et al. 2008). Foraging trails outside the structure in the surrounding landscape, on utility lines, or on the trunks of trees should also be sprayed. Trees in which the ants may be nesting are also target areas for treatment (Klotz et al. 2008).

Access into the structure should be eliminated by trimming trees and shrubs that are in contact with the structure or wires and cables leading into the structure (Klotz et al. 2008). Entry points around windows, doors and fixtures should be sealed.

Ants located in dead wood are best eliminated by removing infested wood; but this is not always possible. Another treatment option is to drill and inject a small amount of insecticide dust labeled for this application into the galleries. Ants located in firewood can be eliminated by discarding or burning the infested wood. Never treat firewood with residual insecticide (Hedges 1998)! Ants located in the soil under rocks and stones can be treated by thoroughly drenching the nest with residual insecticide (labeled for such use) using a compressed air sprayer (Hedges 1998).

SUMMARY

Members of the genus *Liometopum* are closely associated with trees and shrubs. Even though *L. luctuosum* and *occidentale* have been reported as structural pests in California, Oregon, and Washington, very little is known about their biology. Both species are often misidentified and confused with carpenter ants. Additional research on
these species is warranted, especially the foraging behavior and feeding preferences, colony structure and intracolony interactions, and their physiological ecology.
REFERENCES


Table 1: Colony foundation of *L. apiculatum* modified from Conconi et al. (1983a)

<table>
<thead>
<tr>
<th>Event</th>
<th>Time (days)</th>
<th>Event</th>
<th>Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass Tubes</td>
<td></td>
<td>Jar with Soil</td>
<td></td>
</tr>
<tr>
<td></td>
<td>32°C/ 70-80% RH</td>
<td>26°C/ 40-50% RH</td>
<td></td>
</tr>
<tr>
<td>First eggs</td>
<td>11 ± 2.3</td>
<td>First eggs</td>
<td>27.8 ± 6.9</td>
</tr>
<tr>
<td>First pupa</td>
<td>9.61 ± 2.1</td>
<td>First pupa</td>
<td>24.2 ± 6.2</td>
</tr>
<tr>
<td>First larva</td>
<td>9.84 ± 2.4</td>
<td>First larva</td>
<td>25.16 ± 7.8</td>
</tr>
<tr>
<td>First adults</td>
<td>28.23 ± 4</td>
<td>First adults</td>
<td>70.83 ± 11.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>28.23 ± 5</td>
<td><strong>Total</strong></td>
<td>70.83 ± 11.4</td>
</tr>
</tbody>
</table>
Figure 1: Insulation removed by *L. luctusosum* in a mountain home in the San Bernardino Mountains, CA.
Figure 2: Damage to wood caused by workers of the *L. luctuosum*. 
Figure 3: A - Frass produced by *L. luctuosum*. B – frass produced by the carpenter ant, *Camponotus modoc*.

A)

B)
Chapter 2:

Food and bait preferences of *Liometopum occidentale* (Hymenoptera: Formicidae)
ABSTRACT

The velvety tree ant, Limetopum occidentale Emery, is commonly found in urban areas throughout the western U.S. and has been reported damaging structures. Foragers prefer sucrose, glucose and honey sucanat solutions. Solid protein baits containing anchovy were also retrieved by workers. In the early summer, foragers were active both day and night. In the late summer when daytime temperatures exceeded 35 °C, workers foraged at night. Even though workers are polymorphic, they all consumed about 0.25 mg of a 25% sucrose solution and thus providing a mechanism of determining foraging activity by sugar water removal from monitoring stations. Liquid bait bases containing 25% sucrose would be effective if suitable toxicants can be identified.

INTRODUCTION

The velvety tree ant, Liometopum occidentale Emery, is commonly found along the coastal regions from southern Washington to northern Mexico (Del Toro et al. 2009, Snelling & George 1979, Dr. Laurel Hansen personal communication). This ant can be found at elevations as low as 7 m in Oregon to over 1700 m in California (Dr. Laurel Hansen personal communication; personal collection). They are the most common and dominant ant in oak and pine forests of southwestern U.S. (Wheeler & Wheeler 1986, Ward 2005, Del Toro et al. 2009). They prefer to nest in the crevices of oaks, alders, elms, cottonwoods, and creosote, and in soil, underneath bark of dead trees, and under rotten logs (Cook 1953).
*Liometopum occidentale* is also commonly found in urban areas of southern California. Structural damage caused by *L. occidentale* has been reported (Wheeler & Wheeler 1986, Merickel & Clark 1994, Gulmahamad 1995, Hedges 1998, Klotz et al. 2008). However, velvety tree ants are often mistaken for carpenter ants (*Camponotus* spp.) by homeowners and Pest Management Professionals (PMPs). This mistaken identity is due to morphological and behavioral characteristics they share with carpenter ants, namely polymorphic workers, a smooth convex thoracic profile, and the tendency to excavate wood (Klotz et al. 2008). Consequently, their importance as structural pests may be greatly under reported, especially in California, Oregon, and Washington.

*Liometopum occidentale* form massive foraging trails over 60 m in length with some trails as long as 145 and 185 m (Conconi et al. 1983a,b; Gulmahamad 1995) and covering areas up to 740 m² (Del Toro et al. 2009, Wang et al. 2010). Workers travel mostly underground in very shallow galleries (1-2 cm deep), or in the litter (Ramos-Elorduy & Levieux 1992). Once they invade oak trees, they will use this resource indefinitely. Foragers forage at temperatures between 24 – 38 °C (Tremper 1971). *Liometopum occidentale* forage during the day in great numbers in the spring and early summer, but around mid-summer they switch to nighttime foraging, likely due to increasing daytime temperatures. This has been observed, but not experimentally tested. They are opportunistic omnivores (Wheeler 1905) and can often be found tending hemipterans and carrying prey insects back to the nest (Conconi et al. 1987, Gulmahamad 1995, personal observations).
Recently there has been renewed interest in the use of baits as a means of ant control because baits deliver low volumes of toxicants directly to target species, are easy to apply, require no mixing by the applicator and can be used against ants that nest in protected harborages and are often unaffected by sprays (Klotz et al. 2000, 2008, 2009; Hooper-Bui & Rust 2000). In addition, conventional perimeter sprays can generate unintended pesticide runoff in urban water ways (Greenberg et al. 2010). Toxic baits are generally considered to be more effective in controlling ant infestations than insecticidal sprays and dusts because they are more likely to eliminate ant colonies by killing queens and brood, as well as foraging workers (Williams et al. 1990, Knight & Rust 1991, Williams & Vail 1993). However, baits need to be highly acceptable to ants to compete for the attention of foragers if other food sources are available (Cornelius et al. 1996). In order to develop an attractive bait, the food preferences of the pest ant must be determined.

Two commonly used methods of determining food preference are (1) to count the number of ants attracted over time to various foods offered simultaneously, or (2) to measure the amount consumed of various foods offered simultaneously and compared the proportions of the total taken. In this study, we used the second method because it provides an estimate of the amount of bait base that is actually retrieved by foragers. For example, Argentine ants are attracted to sucrose gels however, the handling time of this food is so great due to its consistency that the ants do not consume as much of it as a liquid sucrose solution (Silverman & Roulston 2001). The second approach provides a
better estimate of foraging activity over time, especially for species that might only
forage sporadically or during specific times of the day.

Control of *L. occidentale* is problematic because of the distance that workers
forage and that nests may not be located on the property being treated. Consequently,
baits are a reasonable alternative to spraying pesticides if suitable baits could be
developed. The objectives of this study were to (1) develop a suitable choice feeding
bioassay, (2) determine if there are daily patterns of foraging behavior, (3) determine if
there are preferred foods that might serve as bait bases for toxic baits, and (4) determine
an effective method of estimating foraging activity.

**MATERIALS AND METHODS**

**Food preference studies**

Food preference studies of *L. occidentale* were conducted in the field with choice
tests because it was impossible to maintain large natural laboratory populations. During
the choice tests, pre-weighed open vials of different candidate types of food were placed
within a foraging arena near the ants’ foraging trails. The candidate foods were left out
for a fixed time interval then returned to the laboratory and reweighed. An arena designed
by Rust et al. (2000) was used for these experiments (Fig. 4). The arena consisted of an
aluminum cake pan (20 cm diam. by 4 cm high) with 4 openings set 90° apart. Pieces of
glass tubing (7 cm long by 7 mm outer diam.) were inserted through the openings so the
ends were flush with the outside of the pan. This directed to ants towards the center of the
arena. Exactly 5 ml of liquid or 3 g of solid food material was measured out into 14.8 ml
glass vials with screw cap lids. The vials were opened and placed in a random pattern within the arena. The arena was covered with a piece of transparent mylar plastic and a piece of plywood covered with aluminum foil to provide shade and protection from rodents and other animals. Ant foraging activity was assessed by determining the amount of food removed. The amount of food removed (initial food - final food weight) was corrected by the percent reduction or gain in weight due to evaporation or absorption of water in the control foods placed simultaneously in a protected outdoor location where the ants were unable to forage on them. The amount of bait removed was corrected by the percentage of reduction or gain in weight of the control bait with following formula:

\[
\text{(Test Food}_{\text{initial}})\ (\text{Evap Food}) - (\text{Test Food}_{\text{final}}), \text{ where Evap Food} = \text{Evap Food}_{\text{initial}} - \text{Evap Food}_{\text{final}}, \text{ in the controls. If Evap Food}_{\text{initial}} - \text{Evap Food}_{\text{final}} < 0, \text{ then multiply by -1 then add 1 to Evap Food.}
\]

Data were collected as two sets. Set one was tested on days in July 2010 (July 5-31) when temperatures ranged between 19.1-27.1°C (66.3-80.8°F), and at night when temperatures ranged between 16.4-24.2°C (61.6-75.6°F). Data set two was collected on nights in September 2010 and August 2011 (September 25-26 and July 27-August 23) when temperatures ranged between 18.4-28.6°C (65.1-83.4°F).

A variety of solid and liquid foods that contain sugars, fats and/or proteins was tested in the first test. Six food choices (25% glucose, 25% sucrose, 25% honey sucanat, anchovy granules, roach granules, earthworm granules) were tested during 6 h trial periods during the day (10a.m.-4p.m.; or approx 4 h after sunrise until approx 4 h before sunset; n=17) and at night (7p.m.-1a.m., approx 1 h before sunset and approx 5 h before
sunrise; n=18). In the second test, nine choices (same six as above plus 25% agave nectar, 25% fructose, and water) were tested during 12-h trial periods (7p.m.-7a.m., or at approx. sunset until approx. sunrise; n=24).

To ensure that consumption of foods in the choice pans was only from *L. occidentale*, I only included pans in which *L. occidentale* were found at the end of the test period.

**Food preparation**

The liquid carbohydrate baits were prepared from sucrose (Fischer Scientific®, Fair Lawn, NJ), D- (+)-glucose (Sigma®, Sigma-Aldrich Co., St. Louis, MO), D-(−)-fructose (Sigma®, Sigma-Aldrich Co., St. Louis, MO); honey sucanat / honey crystals (The Prepared Pantry®, Rigby, ID, a mixture of evaporated sugar cane juice and honey). The liquid agave nectar (Madhava®, Madhava Honey, Lyons, CO) was prepared by measuring 30.5 ml of the syrup (as it already contained 18% water) into a graduated cylinder, and was filled with 69.5 ml distilled water to provide a 25% agave nectar solution. All the solutions were stirred until clear, and stored in the refrigerator until needed. Twenty-five percent solutions (wt/vol) were prepared because this concentration was highly preferred by *Linepithema humile* (Mayr) (Baker et al. 1985, Rust et al. 2000) and *Solenopsis invicta* Buren (Greenberg et al. 2004).

The granular bait bases were prepared by following a prescribed recipe according to (Hooper & Rust 1997). Animal material such as anchovy (packed in oil), American cockroaches, and earthworms were ground up (50 g), and mixed with sugar, salt, water,
powdered dry eggs and corn grit (150 g) to produce a paste. The paste was then freeze dried for 24 to 48 h. The bait is then gently loosened into individual granules. The addition of cornmeal and anchovy significantly improved the acceptance of the diet by the southern fire ant, Solenopsis xyloni McCook, than over containing mealworms or beef hash. This addition also resulted in more uniform particle size. The addition of powdered dried eggs was found to be attractive to southern fire ant, and reduced the time required to freeze-dry the diets (Hooper & Rust 1997).

Individual consumption

To determine the amount sucrose solutions consumed by workers in a 30 min period, groups of approx. 20 L. occidentale workers from 3 different colonies were starved for 72 h in 237 ml (8oz) polystyrene cups (hi-plas) coated on the inside with Teflon T-30B (Dupont) to prevent them from climbing the cup. Each cup was provided a moist cotton ball and covered with a lid to prevent dehydration. This set-up maintained a relative humidity within the cup of approximately 80%. This is extremely important because ants exposed in open cups during the starvation period died and the few survivors had extremely high intake of 25% sucrose water. Ants were then anesthetized with CO₂, separated with one ant per ~ 60 ml (2oz) salsa cup, which was coated on the inside with Teflon T-30B (Dupont) to prevent ants from climbing the walls of the cup and covered with a lid. In order to determine their pre-feeding weight, individual ants were then anesthetized with CO₂, weighed to 0.1 µg using a Satorius M2P balance (Sartorius AG, Goettingen, Germany), and returned to their individual cups. After
recovering, they were provided 25% sucrose water for 30 min, anesthetized with CO₂, and weighed again to determine their post-feeding weight.

To determine the effects of starvation and holding ants without water, several groups were tested. Group one consisted of ants from one of the three laboratory colonies that were starved for 72 h without a lid on the cup, and fed for the 30-min period. Test group 2 consisted of ants from colony one that were starved for 72 h but not fed during the 30-min ‘feeding’ period between measurements. Group 3 consisted of ants from colony one that were not starved (taken directly from the colony box) and held with 25% sucrose water for 30 min. Lastly, ants from group 4 were from colony one that were not starved (taken directly from the colony box) and not fed during the 30 min ‘feeding’ period.

**Statistical Analyses**

Data for each food within each arena were converted into proportions of total take from each arena to adjust for potential differences in the size of ant colonies. The data from these choice tests were analyzed using the Friedman’s test of rank sums and multiple comparisons using SYSTAT. To be effective, choice feeding studies require relatively large samples. At least three times as many replicates as choices was recommended by Williams and Titus (1988) after simulating the study stability of variable loadings in linear discriminant analysis (which is structurally similar to multivariate analysis of variance). Roa (1992) then extended these recommendations to both parametric and nonparametric analyses of multivariate food-preference such as
Friedman’s test of rank sums. Nonparametric approaches usually need even more replicates than the parametric procedure because of the reduction in statistical power inherent in using ranks instead of raw data. Furthermore, the statistic T only approximates the F distribution, and does it improve as the sample size increases. Paired t-tests (SYSTAT) were used to compare consumption of foods during the day versus during the night, as well as the consumption of carbohydrates versus proteins during the July trials.

The mean weights before and after feeding, and weight gain (after feeding weight - before feeding weight = consumption) were analyzed with an ANOVA. To determine the relationship between body size and sucrose consumption, Pearson’s correlation coefficient was used to compare their initial weight and their weight gained after feeding (consumption) of the three experimental colonies pooled together. Statistics were performed using R version 2.14.1 (Copyright© 2011 The R Foundation for Statistical Computing).

RESULTS

Food Consumption

In the July trials, cockroach and earthworm granules were the least consumed bait bases by L. occidentale during daytime and consumption was significantly less than the liquid carbohydrates (Fig. 5). The consumption of solid anchovy bait base and the glucose and sucrose solutions was not significantly different. The liquid sugar solutions were the most consumed. During the nighttime trials in July, the cockroach and worm
granules were the least consumed bait bases, with the consumption of cockroach granules being significantly different from that of the carbohydrates and worm granules being significantly different from carbohydrates with the exception of honey sucanat (Fig. 6). Granular anchovy bait base was the most consumed protein bait base during nighttime and daytime foraging, and was not significantly different from liquid carbohydrate bait bases during nighttime foraging time (Fig. 6). Overall, *L. occidentale* consumed more liquid bait bases containing sucrose, glucose, and honey sucanat, with the exception of anchovy granules at night, and did not show a significant difference in consumption based on time of day (Kruskal-Wallis: $\chi^2 (1) = 1.412$, $P = 0.2348$). When there is only a small difference between certain bait bases, it takes a lot of replicates to provide clear separation.

During the September trials, the ants were moving around during the hottest periods of the day, but would not enter the arenas; therefore all trials were conducted at night. Water was also added as a choice to act as a control to determine if the consumption of the liquid carbohydrates was due to the sugars and not just the water used to desolve them. *Liometopum occidentale* showed the similar consumption of carbohydrates during these trials as was seen during the July trials (Fig. 7).

When the proportions from all the carbohydrate solutions consumed were pooled together and the same is done with the proteins, the results showed that *L. occidentale* consumed significantly more of liquid carbohydrate solutions than proteins both during the day and at night (Paired $t$-test: July Day: $t=4.93$, $P=0.0002$; July Night: $t =2.49$, $P=0.0233$). When this same procedure was used for the September trials, *L. occidentale* also
consumed more of liquid carbohydrate solutions than both proteins and water (Friedman’s test: $X^2 (8, 23)= 81.63662, P< 0.0001$). The consumption of carbohydrates was significantly different from that of the proteins ($t = 3.8006, P= 0.00042$) and water ($t = 8.63775, P< 0.0001$). The consumption of the protein bait bases was also significantly different from that of water ($t = 4.8371, P< 0.0001$).

**Individual consumption**

*Liometopum occidentale* workers are polymorphic and their weights are normally distributed (Fig. 8, Shapiro-Wilk Normality Test, $P= 0.9749$). However, not all colonies had the same size distributions.

Starving ants for 72 h in a cup without a lid and then feeding them for 30-min period resulted in high mortality during the starvation period (only colony 3 contained enough live ants to weigh for this experiment) and very high sugar consumption during the feeding period. When comparing ants held in cups without lids to those with lids, the mortality of the ants in cups with lids group with lids on the cups was significantly lower than ants with cups without lids. The high mortality was most likely due to desiccation.

The mean consumption of ants starved for 3 d is $0.258 \pm 0.0528$ (mean ± standard error) mg of 25% sucrose water. The mean weights of the three colonies before feeding were statistically different ($F_{2, 42} = 6.9202, P= 0.0025$) and therefore cannot be pooled together for further analysis. The mean weights after feeding of the three colonies were also statistically different ($F_{2, 42} = 5.0998, P= 0.0104$) and therefore cannot be pooled together for further analysis. However, the mean weight gain or consumption per ant ($F_{2,}$
of the 3 colonies were not statistically different; therefore the colonies were pooled together in further analyses of the data.

There was no relationship between the ant’s initial weight before feeding and the amount of 25% sucrose water consumed ($t = -1.4217$, $df = 43$, $P = 0.1623$, $R = -0.2119$, Fig. 9). All *L. occidentale* workers, regardless of their initial weight (“size”) consumed the same amount of 25% sucrose water.

**DISCUSSION**

The choice feeding assay developed by Rust et al. (2000) was effectively modified to determine the food preferences for *L. occidentale*. Once worker ants find acceptable foods, they initiate recruitment to that particular food source. The quantity of food removed is dictated by the quality and the ease at which workers can handle or process that particular food item. Silverman and Roulston (2001) found that *L. humile* removed greater amounts of liquid sucrose compared with the same amount of sucrose in gel form because of a shorter handling time. Similarly, Hooper and Rust (1997) found that particle sizes of diet of 840-2,000 µm resulted in the largest amount of diet removed by *S. xyloni* foragers.

Liquid and solid bait bases were tested because they are utilized by different members in the colony. Liquids are consumed by workers or carried back to the nest by storing it in her crop (Klotz et al. 2008) and then regurgitating the food to other workers, larvae or the queen, via trophallaxis. Ant foragers are not capable of swallowing solid food particles because they have a constricted esophagus and a filter consisting of ridges
and hairs that line the infrabuccal cavity that prevent particles beyond a certain size from passing into the gut. The threshold size of particles that cannot be ingested by adults varies between species (Klotz et al. 2008), but are always extremely small. For example, *Camponotus pennsylvanicus* (DeGeer) ingest particles smaller than 100 μm in diameter (Klotz et al. 2008), while *Solenopsis invicta* Buren filter out particles as small as 0.88 μm (Glancey et al. 1981). Solid baits with particles larger than the threshold size for ingestion by workers are collected and fed to the larvae that have the ability to digest solids (Klotz et al. 2008). Therefore, the targets of these foods are usually only the larvae.

A wide variety of food bait bases have been tested against various species of ants and we selected several of the more attractive and preferred foods. Twenty percent sucrose and honey solutions were particularly attractive to *Tapinoma indicum* (Forel) and of the proteinaceous foods tested tuna was preferred (Chong & Lee 2006). They did not feed on oils. Choice tests with white-footed ants, *Technomyrmex albipes* (F. Smith), found that 25% sucrose solutions and an artificial nectar-honeydew were highly preferred (Warner & Scheffrahn 2004). Tinti and Nofre (2001) found that *Lasius niger* preferred melezitose > sucrose = raffinose > D-glucose = maltose = sorbitol. *Linepithema humile* preferred 25% honey water and sucrose over solid sugars and other solid foods with protein content (Rust et al. 2000, Baker et al. 1985). Foods that have been tested on *Ochetellus glaber* (Mayr), *Paratrechina longicornis* (Latr.), and *Pheidole megacephala* (Fab.) include but are not limited to canola oil, corn oil, olive oil, peanut oil, safflower oil, soybean oil, fructose, glucose, maltose, melizitose, sucrose, and trehalose (Cornelius et al. 1996). *Ochetellus glaber* significantly preferred sucrose over maltose, but showed
little preference for the oils. *Paratrechina longicornis* did not show a preference between the sugars or between the oils. *Pheidole megacephala* showed a significant preference for melezitose over glucose, maltose, and trehalose, but not over fructose and sucrose, and also showed a preference for olive oil (Loke & Lee 2004). Results indicated that this species preferred protein-rich food (peanut butter and dead cockroaches) to those containing only sugar or lipid. The results of these studies indicate that the food type preference of ants in the subfamily Myrmicinae varies greatly even within species, but members of the subfamily Dolchoderine often prefer liquid sugars.

*Liometopum occidentale* showed only a slight food preference difference between day and night trials. Overall food type preference (carbohydrate, protein, or lipid) did not change. Ants consumed more liquid carbohydrates than solid proteins during both day and night trials. This greater consumption persisted in the late summer trials even with the addition of a few more food choices. The exception to this high level of carbohydrate consumption was the anchovy granules, which were consumed at much greater levels during both night trials. This may be due in part to the fact that this food was a mixture of proteins and oils, as the anchovies were packed in oil.

The foraging and food preferences of *L. occidentale* are similar to another North American species *L. apiculatum*. *Liometopum apiculatum* are opportunistic carnivores and granivores (Shapley 1920), feeding on crustaceans, annelids, mollusks, dead vertebrates, animal droppings, and extrafloral nectar (Velasco et al. 2007). However, hemipteran exudates can make up the bulk of their diet in some habitats (Conconi et al. 1983b).
Some ant species such as *Monomorium pharaonis* (L.) and *S. invicta* varied their preference for certain foods with time, weather, caste composition, colony age, long-term feeding history, stage and presence of brood (Edwards & Abraham 1990, Stein et al. 1990). On the other hand, ants such as the *L. humile* and *Tapinoma indicum* Forel did not alternate their preference between food classes (Rust et al. 2000, Chong & Lee 2006). For example, *L. humile* preferred carbohydrate solutions over a one-year period even when proteinaceous foods were provided (Rust et al. 2000).

The types of toxicants that can be used in liquid and solid baits are determined by their solubility. Aqueous sugar solutions require that toxicants such as boric acid, imidacloprid or thiamethoxam to be partly soluble in water. Oil based foods can only be used as baits when incorporated with oil soluble toxicants such as hydramethylnon, methoprene, or pyriproxyfen. Solid protein baits can incorporate toxicants such as fipronil. The anchovy granular bait base is a viable option as a dry proteinaceous bait, since velvety tree ants showed a strong preference for it. This bait would likely be used to target the larvae and queens.

A study by Alder and Silverman (2004) compared four sampling methods for Argentine ants: trailing activity, ant counts at baits, sucrose consumption, and pitfall trap collections. Pitfall traps provided greatest daily variation and were the most time consuming. Sampling variation for the other methods was similar. These researchers recommended worker counts at baits because they required the least amount of time to assess. However, this method only samples the ant population during a short time interval and must be conducted when the ants are most active. Even though *L. occidentale* are
polymorphic and some are much larger ants than *L. humile*, they consumed a smaller proportion of their body weight of sucrose solution (*L. occidentale* 0.17 to 0.5 compared with *L. humile* 1.0). Lower consumption may be explained by the possibility that *L. occidentale* do not have expandable crops. All size workers of *L. occidentale* consumed about the same amount of sucrose solutions (0.258 ± 0.0528 mg). This simplifies estimating the number of ant visits to monitoring stations, a methodology that has been reliably used to quantify the efficacy of ant control treatments with Argentine ants (Reierson et al. 1998, Klotz et al. 2000, Suoja et al. 2000).

Suoja et al. (2000) found that the variation in the number of ants observed based on visual estimates (trail counts) was much lower than those estimated using consumption-based estimates. However, visual counts counted fewer ants and had to be extrapolated to estimate daily ant numbers. Nevertheless, these authors concluded that consumption estimates are unreliable. One reason the authors may have seen such variation in ant numbers using consumption-based estimates is that they used a 10% sucrose solution as their bait for, which is significantly less attractive to Argentine ants than 25% sucrose solution (Baker et al. 1985, Rust et al. 2000). Relative worker numbers on different foods are not always consistent with actual consumption levels, and researchers should be cautious about sole reliance on worker residence at baits as an indicator of bait performance (Silverman & Roulston 2001).
REFERENCES


Figure 4: *Liometopum occidentale* foraging on baits in an uncovered choice arena
Figure 5: Average proportion of the total food taken for each bait type during daytime trials on cool July days in 2010. Error bars represent standard error. Bars with the same letter are not significantly different at p<0.05.

Friedman’s test: $\chi^2 (5, 16) = 26.3070$

$P < 0.001$
Figure 6: Average proportion of the total food taken for each bait type during nighttime trials on cool July nights. Error bars represent standard error. Bars with the same letter are not significantly different at p<0.05.
Figure 7: Average proportion of the total food taken for each bait type during September 2010 / August 2011 trials with error bars representing standard error. Bars with the same letters are not significantly different at P<0.05.

Friedman’s test: $\chi^2(8, 23) = 81.63662$, $P<0.0001$
Figure 8: Histogram of the weight (mg) of 60 ants (from 3 different colonies) that were starved for 72 h.
Figure 9: Weight of 25% sucrose water consumed compared with the ant’s initial weight. Each colony is represented by a different shape.
Table 2: Mean values ± standard error of weights (mg) before and after feeding, weight gain/loss, and the proportion of weight gained or lost in mg.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Before</th>
<th>After</th>
<th>Gain/Loss</th>
<th>Proportion</th>
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<tbody>
<tr>
<td>Starved w/ lid; fed</td>
<td>60</td>
<td>3.3342 ± 0.1712</td>
<td>3.5925 ± 0.1681</td>
<td>0.2583 ± 0.0528</td>
<td>0.0831 ± 0.0158</td>
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<tr>
<td>Starved w/out lid; fed</td>
<td>15</td>
<td>2.9331 ± 0.2039</td>
<td>3.5106 ± 0.2479</td>
<td>0.5775 ± 0.0967</td>
<td>0.2245 ± 0.0408</td>
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<tr>
<td>Starved w/ lid; not fed</td>
<td>15</td>
<td>2.8403 ± 0.1063</td>
<td>2.8122 ± 0.1029</td>
<td>-0.0281 ± 0.0117</td>
<td>-0.0094 ± 0.0040</td>
</tr>
<tr>
<td>Not starved; fed</td>
<td>15</td>
<td>3.3046 ± 0.1240</td>
<td>3.2594 ± 0.1247</td>
<td>-0.0452 ± 0.0042</td>
<td>-0.0141 ± 0.0015</td>
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<tr>
<td>Not starved; not fed</td>
<td>15</td>
<td>3.1095 ± 0.1901</td>
<td>3.0630 ± 0.1885</td>
<td>-0.0465 ± 0.0037</td>
<td>-0.0152 ± 0.0012</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
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Chapter 3:

The Survivorship and Water Loss of *Liometopum luctuosum* and *L. occidentale* Exposed to Different Temperatures and Relative Humidity
ABSTRACT

Two species of Liometopum are commonly found in the western U.S. from Washington to southern California. Liometopum luctuosum is restricted to the coniferous forests in the mountains in the southern range of its distribution, suggesting that it may be less xeric adapted than L. occidentale. Workers of both species are polymorphic. However, workers of L. luctuosum were significantly smaller (median = 1.99 mg) than those of L. occidentale (median = 4.20 mg). When exposed the various temperatures and relative humidities, L. luctuosum workers survived significantly longer than did L. occidentale. Liometopum luctuosum lost significantly more water over 24 h than did L. occidentale at 25.6 and 33.0°C. The cuticular permeability of L. luctuosum was 17.8 and 19.1 at 25.3° and 33°C. The cuticular permeability of L. occidentale was 17.0 and 19.8 at 25.3° and 33°C. The similarity of the CPs and the comparison of the effect of saturation deficit on survival indicated that water loss alone through the cuticle was probably not the major factor affecting survival. Clearly, decreased locomotory behavior and aggregation behavior of L. luctuosum increased survival compared with L. occidentale. Both species are clearly adapted for xeric conditions in the southwest and montaine areas, but also capable of surviving in mesic coastal and xeric climates of the northwest.

INTRODUCTION

There are three of Liometopum, also known as velvety tree ants, found in the western U.S.: L. apiculatum Mayr, L. luctuosum Wheeler, and L. occidentale Emery. L. apiculatum is restricted to the oak-pine-juniper woodlands of the southeast corner of the
U.S., in Arizona, New Mexico and El Paso, Texas from 1512 – 2450 m (Mackay & Mackay 2002; http://www.antweb.org/description.do?genus=liometopum&name=apiculatum&rank=species). The range of *L. luctuosum* extends from temperate habitats in British Columbia, Washington and Idaho, south to Central Mexico and east to Western Texas. This species inhabits pine, oak, Douglas fir, and juniper forests, sagebrush, and high-elevation riparian habitats (Conconi et al. 1987, Clark & Blom 2007). In New Mexico, it is typically found above 2000 m whereas *L. apiculatum* is most commonly found at lower elevations (Mackay & Mackay 2002). Within its range, *L. luctuosum* have been reported at elevations as low as 59 m in Washington (Dr. Laurel D. Hansen, personal communication) up to elevations as high as 2438 m in Mexico (Conconi et al. 1983, Del Toro et al. 2009) and is often strongly associated with but not limited to pine trees (Del Toro et al. 2009). *Liometopum occidentale* is found in lowlands and coastal regions from southern Washington to northern Mexico (Del Toro et al. 2009) at elevations as low as 7 m in Oregon up to 1700 m in locations in California (Dr. Laurel D. Hansen, personal communication). They are the most common and dominant ant in oak and pine forests of southwestern U.S. (Wheeler & Wheeler 1986, Ward 2005, Del Toro et al. 2009). In California, they also are frequently collected in urban settings.

All three species nest under rocks, decaying logs, and in or at the base of trees or large plants (Conconi et al. 1983, Del Toro et al. 2009). *Liometopum occidentale* have also been observed nesting or resting with or without brood in the crotches of oak trees. These shelters are relatively exposed compared to nesting sites of solely terrestrial ants,
but not as exposed as nest sites of arboreal ants. Both *L. luctuosum* and *L. occidentale* readily forage in trees and *L. occidentale* will occupy oak trees and use this resource indefinitely (Tremper 1976). Thus, these ants might be considered to be semi-arboreal. Arboreal ants that live in temperate areas and nest in relatively exposed situations appear to have physiological adaptations to low humidity and are likely to be adapted to temperature extremes as well (Gosswald 1938). *Liometopum luctuosum* and *L. occidentale* may also have similar adaptations as arboreal ants.

Little information is available on the thermal tolerance and survivorship of these ants. Shapely (1920) reported that *L. occidentale* and *L. apiculatum* are active at a wide range of temperatures (8°C - 38°C), humidity (5%-100%), wind, and light. Temperature is the most important factor affecting their activity. For example, the activity of these ants is very low below 15°C, but can increase after a few warm days and with the prospects of food (Shapely 1920). *Liometopum occidentale* have been observed foraging on hot days between 24 – 38°C in arid oak-digger pine woodlands (Tremper 1976). Both *L. luctuosum* and *L. occidentale* have been observed by pest management professionals to forage during the day in great numbers in the spring and early summer, but around mid-summer they switch to nocturnal foraging. However, this has not been experimentally tested.

Physical conditions such as temperature and humidity are usually assumed to play an important role in species distribution at large scales (Addo-Bediako et al. 2000, Krebs 2001, Chown et al. 2002, 2003), while species-level differences in tolerance to physical conditions may promote species coexistence, and in doing so influence species diversity.
at a local scale (Chesson 1986, Tilman & Pacala 1993). Competition may be affected by
abiotic factors. The outcome of interspecific competition is contingent on features of the
physical environment (Park 1954, Connell 1961a, b, Dunson & Travis 1991).
Competitively dominant species, such as *Liometopum*, are commonly limited in their
abundance and distribution more by abiotic factors than by interspecific competition
(Connell 1961a, b; Dunham 1980). In mature colonies, foragers are the individuals most
exposed to the ambient temperature and humidity of the external environment because
the underground colony’s microenvironment, where non-foragers live, is carefully
selected and controlled (Hölldobler & Wilson 1990). This makes foragers the ideal caste
to study the effect of abiotic factors on the various aspects of their biology, including
survival.

The more wide spread distribution of *L. occidentale* at lower elevations,
especially in southern California and Mexico, suggests that these species might be more
xeric adapted than *L. luctuosum*. The objectives of the study were to determine if
environmental factors such as temperature and relative humidity might affect the
distribution of each species. Live workers of both species were exposed to combinations
of three different temperatures and relative humidity likely to be encountered by each
species. In addition, the cuticular permeability and water loss rates of both species were
determined. Their implications for the distribution of each species are discussed.
METHODS AND MATERIALS

Ant Collection

Ant foragers were collected in plastic boxes (12 cm by 8 cm by 6 cm) coated on the inside with Teflon T-30B (E.I. du DuPont Nemours and Co., Wilmington, DE) to prevent them from escaping. Ants were collected by gently brushing foragers off the trunk of the nest tree using a soft bristled paint brush into the plastic boxes. Ants were taken to the University of California, Riverside, where they were maintained in the laboratory in the boxes in which they were collected. Ants were provided harborage consisting of a Petri dish (14 cm in diameter) that was half filled with plaster of Paris, as well as, a continuous supply of water and 25% sucrose water. Three times weekly, pieces of dead American cockroaches, Periplaneta americana L. were placed in the boxes.

Three different colonies of each species were used in these experiments. Data concerning the collection sites are listed in Table 3 (Fig.1). The collection sites in San Bernardino Co. experienced temperatures as low as – 8º C (18º F) in winter and as high as 37º C (98º F) in the summer. The collection sites in Riverside Co. experienced temperatures as low as 0º C (32º F) in winter and as high as 44º C (112º F) in summer. The distance between L. occidentale colonies 1 and 2 is 4.0 km, colonies 1 and 3 is 30.2 km, and colonies 2 and 3 is 32.1 km. The distance between L. luctuosum colonies 1 and 2 is 3.9 km, 1 and 3 38.2 km, and 2 and 3 is 42.1 km.
Exposure to Various Temperatures and Relative Humidity

*L. occidentale* and *L. luctuosum* workers were held in groups of 20+ in 237 ml polystyrene cups (hi-plas®) coated on the inside with Teflon T-30B to prevent their escape. Each group was provided water and 25% sucrose water the day before ants were selected for the experiments (18 h) to try to ensure that all ants were well fed and hydrated. Once they were randomly selected for the experiment they were no longer provided food or water. Three different levels of relative humidity were maintained in glass desiccator jars (with saturated salt solutions of magnesium chloride (32% RH), sodium dichromate (52% RH), and sodium chloride (75% RH) (Winston & Bates 1960). The humidity chambers were then placed in three different environmental chambers maintained at 15.6, 23.9 and 32.2°C. The number of dead ants was recorded every 12 h up to 216 h.

Cuticular Permeability (CP) and Water Content

The cuticular permeability and body water content of *L. occidentale* and *L. luctuosum* were determined gravimetrically. Laboratory colonies were provided water and 25% sucrose solution the day before ants were selected for inclusion in this study (18 h) to try to ensure ants were well fed and hydrated. Ants were then selected from laboratory colonies and held overnight (12 h) with only water in a covered 237 ml cup coated on the inside with Teflon. This design maintained ants at 80% RH. The ants were then killed by exposing them for 1 h in a KCN killing chamber in still air. Ants were removed from the chamber and individually weighed to the nearest 1 µg (Sartorius M2P
balance) and placed in polystyrene weighing boats (2.54 by 3.81 cm). Twenty weighing boats of ants were placed in an 11-liter desiccator chamber containing about 0.7 kg anhydrous CaSO₄ (Drierite®). The unstimred air in the chambers was maintained at 0-2% RH. The Drierite was conditioned by heating it for 4 h at 204°C to remove all water. The conditioned desiccators were stored at 25.3° and 33°C for 24 h to ensure each chamber was stabilized when specimens were introduced. Temperatures selected represent two temperatures to which both species might be exposed in the field. The specimens were weighed at 2, 4, 6, 8, and 24 h and placed back into the chamber.

The percent body water content for each species was determined by calculating the difference between the fresh and dry weight. After the 24 h exposure, dry weights were determined by moving all of the chambers to an environmental chamber maintained at 33°C. Specimens were weighed at day 7 and day 8 and the final weights averaged. Weight loss was assumed to be entirely due to the loss of water.

Cuticular permeability is usually expressed as µg of water lost cm²/hr/mm Hg and was calculated for each ant as the difference in successive weights (wt₁-wt₂) divided by surface area (cm²), time (hr), and saturation deficit (mmHg):

\[ CP = \frac{(wt₁ - wt₂)}{cm²/hr/mm Hg} \]

Typically surface area (SA) is calculated from the fresh weight of the animal (wt in grams) by Meeh’s formula in which \( SA = k \ (wt)^{2/3} \) where k represents a species or group specific constant (Edney 1977). In our study we used three different published methods of estimating surface area. Appel et al. (1983) used of k=12 to calculate SA for cockroaches and other insects. We also used a formula from Haagsma et al. (1996), in
which \( SA = 0.0886 + 26.85(\text{wt}) - 214.21(\text{wt})^2 \) and \( \text{wt} \) equals the insect’s weight in grams. We also estimated using a formula from Lighton & Feener (1989) in which \( SA = k(\text{wt})^{0.667} \) and \( \text{wt} \) equals insect weight in mg. In this formula \( k = 0.103 \) which was determined empirically by measuring the ant *Pogonomyrmex rugosus* Emery. The saturation deficit of the chambers at 25.3° and 33°C were 23.8 and 37.7 mm Hg, respectively. Typically the greatest water loss is experienced in the first 2 h and this was used to determine CP (Appel et al. 1983).

**Statistics**

The benefits of utilizing survival analyses are that you can compare the groups’ survivorship curves over all event times, make predictions by comparing hazard functions, compare shapes of distributions, and account for censored data. When evaluating time until-an-event data in a time-limited experiment, some individuals do not exhibit the event (death) within the time set for the experiment. Such observations are termed “censored” or truncated observations (Cox & Oakes 1984). In this experiment, data were considered censored if death did not occur by the time the experiment was terminated (216 h).

Survival curves for all the covariates (temperature, humidity, species and colony) were estimated with the Kaplan-Meier technique and were compared using a log rank test with a chi-square approximation (SigmaPlot® 12). Multiple comparisons were conducted using Holm-Sidak (SigmaPlot® 12).
A Cox Proportional Hazard Regression (CPHR) model was used to determine the relationship between mortality (response variable-y) and relative humidity, temperature, and species (fixed factors) using SAS 9.2 statistical software. This type of model allows for predictions of the effect of various levels of these factors on the hazard function and therefore the survivorship of these ants. The modification of the effect of the variables was evaluated by including interaction terms in the model. Because the species are significantly different in their hazard functions, CPHR models were created for each species. Model selection was conducted using a forward selection method in SAS 9.2, in which model terms were added one at a time until the model was significantly different from the previous model, when this occurred the previous model was used as the final model. Overall model significance was determined using a Likelihood Ratio test.

RESULTS

Survival

Comparisons of Both Species Overall

Using Kaplan-Meier Log-Rank Survival Analysis to graph and analyze the effect of each variable separately on the survival of Liometopum, we found that the survival of L. luctuosum was significantly greater than that of L. occidentale (Log-rank: \( \chi^2 = 141.5919, \text{DF}=1, P < 0.0001 \)). The survival curves of both species pooled together under each temperature were significantly different (Log-rank: \( \chi^2 = 1038.8037, \text{DF}=2, P < 0.0001 \)). The survival curve for 15.6°C (60°F) had a much shallower slope than did the curve for 24.0°C (75°F) (Holm-Sidak: P< 0.0001) which was shallower than was 32.2°C
(90°F) (Holm-Sidak: P< 0.0001). In other words, as temperature increased, the rate of survivorship decreased dramatically (Fig. 11a). The survival curves of both species combined under each humidity were significantly different (Log-rank: $\chi^2 = 57.6547$, DF= 2, P < 0.0001). The curve for 75% RH had a slightly shallower slope than the curve for 52% RH (Holm-Sidak: P= 0.0935) which was shallower than 32% RH (Holm-Sidak: P< 0.0001). In other words as relative humidity decreased survival decreased (Fig. 11b).

The CPHR model which includes both species and all the variables was as follows: $Time*Dead(0) = Temperature + Humidity + Species + Species(Colony) + Temperature*Humidity$ (Likelihood Ratio $\chi^2 = 1715.5165$, DF= 13, P< 0.0001). Even using this model, the survival rate of *L. luctuosum* was significantly greater than that of *L. occidentale* (Table 4; $\chi^2 = 543.0314$, DF= 1, P< 0.0001). The differences between the levels of temperature remained the same in this model as they were using the Kaplan-Meier LogRank Analysis (CPHR: 15.6 vs. 23.9°C $\chi^2 = 394.4895$, P< 0.0001; 15.6 vs. 32.2°C $\chi^2 = 1054.4327$, P< 0.0001; 23.9 vs. 32.2°C $\chi^2 = 627.2503$, P< 0.0001). The differences in the levels of humidity did not remain the same in this model as they were with the Kaplan-Meier LogRank Analysis (Table 5). Using this model, the 52% and 75% levels of humidity were significantly different (Table 5: 52 vs. 75 $\chi^2 = 41.0777$, DF= 1, P< 0.0001).

*Liometopum luctuosum*

Using Kaplan-Meier Log-Rank Survival Analysis to graph and analyze the effect of each variable separately on the survival of *L. luctuosum*, survivorship increased as the
temperature decreased (Log-rank: $\chi^2 = 781.8358$, DF= 2, $P< 0.0001$) and survivorship decreased as the relative humidity decreased (Log-rank: $\chi^2 = 35.7713$, DF= 2, $P< 0.0001$).

Each level of temperature was significantly different from each other (Holm-Sidak: $15.6$ vs. $23.9^\circ C$, $P< 0.0001$; $15.6$ vs. $32.2^\circ C$, $P< 0.0001$; $23.9$ vs. $32.2^\circ C$, $P< 0.0001$). Each level of relative humidity was significantly different from each other, with the exception of $52\%$ vs. $75\%$ (Holm-Sidak: $32$ vs. $52$ $P< 0.0001$, $32$ vs. $75$ $P< 0.0001$, $52$ vs. $75$ $P= 0.775$). There were significant differences between the survival curves of the different colonies of *L. luctuosum* (Fig. 12; Log-rank: $\chi^2 = 25.7916$, DF= 2, $P< 0.0001$). The survival of colony 1 was significantly different from colonies 2 and 3, but colonies 2 and 3 were not significantly different from each other (Holm-Sidak: $1$ vs. $2$ $P= 0.000305$, $1$ vs. $3$ $P< 0.0001$, $2$ vs. $3$ $P= 0.319$). The survival rates of the colonies of *L. luctuosum* can be ordered from highest to lowest as follows: 3, 2, 1.

The CPHR model which includes *L. luctuosum* and all the variables was as follows: $\text{Time*Dead(0)} = \text{Temperature} + \text{Humidity} + \text{Colony} + \text{Temperature*Humidity}$ (Likelihood Ratio $\chi^2 = 903.9162$, DF= 10, $P< 0.0001$). The relationship and differences between the levels of temperature remained the same in this model as they were with the Kaplan-Meier LogRank Analysis (CPHR: $15.6$ vs. $23.9^\circ C$ $\chi^2 = 176.7696$, $P< 0.0001$; $15.6$ vs. $32.2^\circ C$ $\chi^2 = 553.209$, $P< 0.0001$; $23.9$ vs. $32.2^\circ C$ $\chi^2 = 335.7578$, $P< 0.0001$). The relationship of relative humidity and survivorship remained the same as those found with the Kaplan-Meier LogRank Analysis (Table 5). However, the differences in the levels of relative humidity did not remain. Using this model, the $52\%$ and $75\%$ levels of humidity were significantly different (Table 5: $52$ vs. $75$ $\chi^2 = 9.9217$, DF= 1, $P= 0.0016$). Unlike the
results from the Kaplan-Meier LogRank Analysis we found that the survival rates of all three colonies of *L. luctuosum* were significantly different from each other (Table 5; 1 vs. 2, $\chi^2 = 32.5779$, DF= 1, P < 0.0001; 1 vs 3, $\chi^2 = 64.5286$, DF= 1, P < 0.0001; 2 vs. 3, $\chi^2 = 5.8122$, DF= 1, P = 0.0159) using the CPHR. The survivorship of the colonies can be ordered from longest survival to shortest survival as follows: 3, 2, 1.

*Liometopum occidentale*

Using Kaplan-Meier Log-Rank Survival Analysis to graph and analyze the effect of each variable separately on the survival of *L. occidentale*, survivorship increased as the temperature decreased (Log-rank: $\chi^2 = 502.6529$, DF= 2, P < 0.0001) and survivorship decreased as the relative humidity decreased (Log-rank: $\chi^2 = 32.4506$, DF= 2, P < 0.0001). Each level of temperature was significantly different from each other (Holm-Sidak: 15.6 vs. 23.9°C P = 0.000; 15.6 vs. 32.2°C P < 0.001; 23.9 vs. 32.2°C P < 0.0001). Each level of humidity was significantly different from each other (Holm-Sidak: 32 vs. 52 P = 0.00135, 32 vs. 75 P < 0.0001, 52 vs. 75 P = 0.009). The log rank statistic for the survival curves of the variable “colony” was greater than would be expected by chance and there was a statistically significant difference between survival curves (Fig. 12; Log-rank: $\chi^2 = 111.1179$, DF= 2, P < 0.0001). The colonies *L. occidentale* were significantly different from each other with the exception of colony 1 vs. 2 (Holm-Sidak: 1 vs. 2 P= 0.563, 1 vs. 3 P= 0.0001, 2 vs. 3 P < 0.0001). The survival rates of the colonies of *L. occidentale* can be ordered from highest to lowest as follows: 3, 1, 2.
The CPHR model which includes *L. luctuosum* and all the variables is as follows:

\[
\text{Time} \times \text{Dead}(0) = \text{Temp} + \text{Hum} + \text{Colony} + \text{Temp} \times \text{Hum} \quad (\text{Likelihood Ratio } \chi^2 = 718.4493, \text{ DF} = 10, P < 0.0001).
\]

The differences between the levels of temperature remained the same in this model as they were with the Kaplan-Meier LogRank Analysis (Table 5: CPHR: 15.6 vs. 23.9°C \( \chi^2 = 167.7605, P < 0.0001 \); 15.6 vs. 32.2°C \( \chi^2 = 447.1108, P < 0.0001 \); 23.9 vs. 32.2°C \( \chi^2 = 235.2698, P < 0.0001 \)). The differences in the levels of humidity remained the same in this model as they were with the Kaplan-Meier Log Rank Analysis (Table 5). The survival rates of colony 1 and colony 2 of *L. occidentale* were not significantly different from each other (Table 7, \( \chi^2 = 0.022, \text{ DF} = 1, P = 0.8822 \)), but the survival rate of colony 3 was significantly different from those of colonies 1 and 2 (Table 7, 1 vs. 3 \( \chi^2 = 185.7325, \text{ DF} = 1, P < 0.0001 \); 2 vs. 3, \( \chi^2 = 186.6282, \text{ DF} = 1, P < 0.0001 \)). The survival rates of the colonies can be ordered from highest to lowest as follows: 3, 2, 1.

**Saturation Deficit**

Using Kaplan-Meier Log-Rank Survival Analysis to graph and analyze the effect of saturation deficit on the survival of both species pooled together we found that the nine saturation deficits (at temperatures) can be ordered, from highest survival to lowest survival, as follows: 3.65 (15.56°C), 6.14 (15.56°C), 8.71 (15.56°C), 10.75 (23.89°C), 5.6 (23.89°C), 15.23 (23.89°C), 8.92 (32.22°C), 17.14 (32.22°C), and 24.28 (32.22°C) (Fig. 11d); all are significantly different from each other.
The CPHR model which includes both species and the variable saturation deficit (SD) is as follows: \( \text{Time*Dead(0)} = SD + \text{Specolony} \) (Likelihood Ratio \( \chi^2 = 1715.5165 \), DF= 13, \( P < 0.0001 \); Specolony= Species(Colony)). The survival rates of ants at the nine saturation deficits were significantly different from each other with the exception of SD 6.144 vs. SD 5.6 (Table 7, \( \chi^2 = 0.9162 \), DF= 8, \( P = 0.3385 \)).

**Liometopum luctuosum**

Using Kaplan-Meier Log-Rank Survival Analysis to graph and analyze the effect of saturation deficit on the survival of *L. luctuosum* we found that the nine saturation deficits (at temperatures) can be ordered, from highest survival to lowest survival were as follows: 3.65 (15.56 °C), 6.14 (15.56 °C), 8.71 (15.56 °C), 10.75 (23.89 °C), 5.60 (23.89 °C), 15.23 (23.89 °C), 8.92 (32.22 °C), 17.14 (32.22 °C), and 24.28 (32.22 °C). Only SD 3.65 and 6.14 were not significantly different (Holm-Sidak: \( t = 0.929 \), \( P = 0.335 \)).

When examining the effect of saturation deficit on *L. luctuosum* alone using the following CPHR model: \( \text{model Time*Dead(0)} = SD Colony \) (Likelihood Ratio \( \chi^2 = 899.614 \), DF= 9, \( P < 0.0001 \)); the survival rates of ants at the nine saturation deficits were significantly different from each other with the exception of SD 8.71 vs. SD 3.65 (\( \chi^2 = 0.9256 \), DF= 1, \( P = 0.336 \)) and of SD 6.14 vs. SD 5.60 (\( \chi^2 = 0.5534 \), DF= 1, \( P = 0.4569 \)).

**Liometopum occidentale**

Using Kaplan-Meier Log-Rank Survival Analysis to graph and analyze the effect of saturation deficit on the survival of *L. occidentale* we found that the nine saturation
deficits ordered from highest survival rate to lowest survival rate were as follows: 3.65 (15.56 °C), 6.14 (15.56 °C), 8.71 (15.56 °C), 10.75 (23.89 °C), 5.6 (23.89 °C), 15.23 (23.89 °C), 8.92 (32.22 °C), 17.14 (32.22 °C), and 24.28 (32.22 °C). Only 5.60 and 10.752 were not significantly different (Holm-Sidak: t= 1.715, P= 0.190).

When examining the effect of saturation deficit on *L. occidentale* alone using the following CPHR model: model Time*Dead(0) = SD Colony (Likelihood Ratio $\chi^2=647.2274$, DF= 9, P< 0.0001); the only SDS that were not significantly different were 5.60 and SD 6.14 ($\chi^2= 1.8648$, DF= 1, P= 0.1721), and 6.14 and 24.28 ($\chi^2= 3.437$, DF= 1, P= 0.0638).

**Cuticular Permeability**

Workers of *L. luctuosum* and *L. occidentale* were polymorphic with *L. luctuosum* (median = 1.988 mg; range = 0.910-3.534 mg, n= 38) being considerably smaller than those of *L. occidentale* (median = 4.202 mg; range = 1.437-5.238 mg, n = 39; Fig. 14).

The water loss rates of *L. luctuosum* at 25.3°C were lower than those at 33°C (Fig. 15; 25.3°C, y= 0.028 + 0.025x, $R^2 = 0.9916$; 33°C, y= 0.124 + 0.031X, $R^2 = 0.886$). Similarly, water loss rates for *L. occidentale* were lower at 25.3 °C than at 33 °C (Fig. 15; 25.3°C, y = 0.099 + 0.025x, $R^2 = 0.911$; 33°C, y= 0.885-1.240/x, $R^2 = 0.971$). At both temperatures, the water loss rates for *L. luctuosum* were higher than those for *L. occidentale*. Within 2 h at 33°C, *L. luctuosum* had lost 32.2% of its total body water compared with only 15.9% in *L. occidentale*. 
The CP’s for both species were not significantly different at either temperature (Table 8) when using either SA calculations from Haagsma et al. (1996) or from Lighton and Feener (1989). They lost between 17.0 and 19.8 µg water/cm²/hr/mm Hg. The CP’s were higher for both species at 33°C when determining SA by the constant of 12. *Liometopum occidentale* had significantly higher body water content (68.9%) than did *L. luctuosum* (63.0%) ($F_{37,39} = 1.88$, $P = 0.0274$).

**DISCUSSION**

**Effect of Abiotic Factors**

The distribution of insects on a macro scale is assumed to be influenced by physical conditions of the environment such as temperature and relative humidity (Addo-Bediako et al. 2000; Krebs 2001; Chown et al. 2002, 2003). Environmental factors such as distance to the nearest source of surface water, distance to the nearest edge of the wildlife preserve, mean elevation, mean summer solstice insolation, and slope explained much of the distribution of the invasive Argentine ant and native ant species *L. occidentale* at the hectare scale (Human et al. 1998). *Liometopum occidentale* and *L. humile* were most commonly found close to the edge of the preserve, closer to water, and at lower elevations (Human et al. 1998).

*Liometopum luctuosum* survived significantly longer than *L. occidentale* overall. *Liometopum luctuosum* and *L. occidentale* overlap slightly in the habitats and elevations in which they are found such as the San Bernardino Mts. in southern California. Their typical habitat types are quite different, *L. luctuosum* are typically in coniferous forests
where as *L. occidentale* are typically found in deciduous forests. These habitats differ in precipitation and therefore available moisture, as well as average temperature. Both these species nest amongst the roots of trees, in the crotches of trees, or under the bark of trees, making them “semi-arboreal” ants. *Liometopum occidentale* usually nest in deciduous trees such as oaks, as well as other perennial plants, while *L. luctuosum* usually nest in conifers such as pine. The moisture content of these types of trees differ (http://www.fs.fed.us/ccrc/topics/urban-forests/). The moisture content of trees in general can range from about 30% to more than 200% of the weight of the wood. In softwoods such as pine, the moisture content of sapwood is usually greater than that of heartwood, with moisture content in pines ranges between 31-100% in heartwood and about 106-220% in the sapwood. While, in hardwoods, the difference in moisture content between heartwood and sapwood depends on the species, with the moisture content in oaks ranging between 64-83% in the heartwood and 69-81% in the sapwood. *Liometopum luctuosum* may be nesting in a more moisture rich environment than *L. occidentale.* These two ant species may also differ in the part of the tree in which they nest. Ants that nest in the heartwood would usually experience higher moisture levels versus those that nest in the sapwood, which has lower moisture content. However, it is uncertain in what part of the tree these ants live.

Ants alter their behavior to reduce the risk of death due to abiotic factors both as individuals and as a social unit. During this study *L. luctuosum* appeared to have a greater tendency to aggregate and remained less active than did *L. occidentale.* This aggregation behavior may reduce their rate of water loss, but still remains to be explored.
The ability to reabsorb or produce water would also enable animals with these abilities to survive longer in desiccating conditions. Ants with a larger rectal pad area, for example, have a greater ability to retain fecal water, and therefore can resist death by desiccation for longer periods of time. Rectal pad area is anatomical feature that may affect the survivorship of different ant species (Hood and Tschinkel 1990). Since *L. luctuosum* survived longer than *L. occidentale*, perhaps this is due to rectal pad area. This would be an interesting avenue of study. Production of metabolic water has been detected in *Pogonomyrmex rugosus* Emery in response to low humidity (Lighton & Feener 1989) and would be another interesting phenomenon to explore with ants in this study.

Cuticular hydrocarbons may play an even larger role in water loss rates (Hood & Tschinkel 1990) with lower rate of water loss and lower cuticular permeability resulting in increased survival. However, as you can see from our cuticular permeability results, which are discussed later, we did not observe any differences between these two species.

Body size has been found to have a significant positive effect on the survival of ants, with the survival time of various ant species increasing with increased body size (Hood & Tschinkel 1990, Schilman et al. 2007). This would certainly favor *L. occidentale* which is larger than *L. luctuosum*. We did not find significant difference in the CPs of these species, and even though *L. occidentale* workers were significantly larger overall than those of *L. luctuosum*, they did not survive longer, suggesting that there are other factors that are more important to their survival.

Another explanation may be that *L. luctuosum* have a greater ability to store water and/or liquid food which would increase their water body content, and would in turn
increase their desiccation resistance. An anatomical feature that can enable ants to store excess liquids and may therefore effect the survival of ant species is the expandable crop. The expandable crop is found in many species of Formicines and Myrmicines, but Dolichoderines are best known for this trait. Many of these of Dolichoderines species feed on liquid foods with high water content such as honeydew, giving these ants the advantage of high body water content. In a feeding study, we found that *L. occidentale* consumed 6.73-9.89% of their body weight in sugar water when starved; however, this has not been determined for *L. luctuosum*. Approximately 67-70% of the body weight of *L. occidentale* consisted of water, while approximately 62-65% of the body weight of *L. luctuosum* consisted of water. This, however, does not appear to be significant of a difference to explain the differences in survivorship of these species.

Furthermore, because many abiotic conditions interact, it is possible that the interaction between temperature, humidity and some other abiotic factor(s) such as soil type, radiation, surface water availability, etc. are more limiting to the distribution of *Liometopum* than just temperature and humidity alone. There may also be biotic factors, such as competition for nest sites that are more determinate of distribution of these ants, at least on a local scale.

Increased temperature resulted in increased mortality for both species of *Liometopum*. Holway et al. (2002) for example found that worker survival of invasive Argentine ants and five native ant species was strongly dependent on temperature after a 60-min exposure. Our results corroborate their findings.
Increased humidity in our study resulted in decreased mortality for both species of *Liometopum*. Comparisons by Hood and Tschinkel (1990) 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. Since these ants are semi-arboreal, they would be exposed to additional stress. Hood and Tschinkel (1990) found that survival time of ants in their study increased with increased relative humidity. Even when corrected for body size, they found significant differences in desiccation resistance between species and survival times of different species types (arboreal or terrestrial). Overall, survival times of ants in their study depended on whether the ants were arboreal or terrestrial. Arboreal ants survived 8 times longer than did terrestrial ants of comparable size.

The dryness or wetness of a climate is not correlated with the amount of water vapor in the atmosphere. Saturation deficit (vapor pressure deficit) is an index of humidity typically characterized by the difference between the saturation vapor pressure and the actual vapor pressure of a volume of air. This index has the particular quality of being proportional to the evaporation capability of the air. Saturation vapor pressure increases with increasing temperature, thus the same relative humidity will correspond to a greater saturation deficit at warmer temperatures. Saturation deficit has been found to have an effect on the survival of other arthropods such as isopods (Miller 1938, Warburg 1965a, b, c, White & Zar 1968), Collembola (Joose & Groen 1970), termites (Smith & Rust 1993), and fleas (Silverman et al. 1981). White and Zar (1968) found an increase in temperature, under constant relative humidity, resulted in a decrease in mean survival time of the isopod, *Tracheoniscus rathkei* Brandt. Thus they suggested considering
saturation deficit rather than humidity alone. Similar to our findings Miller (1938) found that temperature appeared to have a greater ecological effect on isopods beyond its influence on saturation deficit. Survival time is inversely related to saturation deficit not just relative humidity in insects that typically live in humid environments such as collembola species, *Tomocerus minor*, *Orchesella cincta* and *Isotoma viridis* (Joose & Groen 1970). However, this relationship is not always found with every insect species. Survival of termite *Reticulatermes hesperus* (Banks), for example, is not correlated with saturation deficit, but instead with the combination of temperature and relative humidity (Smith & Rust 1993). Silverman et al. (1981) also found that there was no relationship between survival and life stage of flea species, *Ctenocephalides felis* (Bouche), and saturation deficit; however, temperature and humidity each had an effect.

The survivorship of both *Liometopum* species at the nine saturation deficits (at temperatures) can be ordered, from highest survival to lowest survival, as follows: SD= 3.65 (15.56°C), 6.14 (15.56°C), 8.71 (15.56°C), 10.75 (23.89°C), 5.6 (23.89°C), 15.23 (23.89°C), 8.92 (32.22°C), 17.14 (32.22°C), and 24.28 (32.22°C) (Fig. 11d). Saturation deficit also has an effect on the rate of water loss of animals. The SDs group according to temperature, not just SD; suggesting that it is simply not just water loss contributing to mortality, but temperature as well. The rate of water loss usually assumed to be directly proportional to saturation deficit of the surrounding air; however, it has been rightfully questioned in regards to the cuticle of insects (Toolson 1978, 1982). In our case, water loss through the cuticle alone is not the critical factor, but instead increased metabolic and behavioral activity may be larger contributing factors to survivorship.
We also found that survival of *L. occidentale* and *L. luctuosum* colonies varied significantly. This is probably because the tolerances of colonies vary slightly between locations and among populations, and suggesting that colonies may be adapting to local conditions. Consequently, it is good practice to use at least three different colonies when conducting studies with social insects such as ants.

**Cuticular permeability (CP)**

Insects have a number of physiological and behavioral adaptations to counteract water loss such as minimizing the surface area to volume ratio, avoiding exposure to high saturation deficits, controlling spiracular activity, depositing specialized cuticular hydrocarbons, reclaiming water, active water sorption and producing metabolic water (Hadley, 1974; Arlian & Veselica, 1979). In Hood and Tschinkel’s study (1990), 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, as well as on the identity of the species within each type. Initial water content of all ants ranged from 60% to 70% and at death from 43% to 60% (Hood & Tschinkel 1990). There was no significant difference in water loss between live and dead ants with arboreal ants losing half as much water as terrestrial ants (Hood & Tschinkel 1990). Increasing water loss has been found to induce active reduction of CP (Hadley, 1972, 1974) or other modes of loss reduction.

Ants lose a majority of their water via the cuticle and only a minor amount is lost via respiration. In fact, respiratory water loss is such a small component of the gross
cuticular permeability it has been calculated to represent only about 10-15% of water loss (Lighton & Feener 1989). Similarly, respiratory exchanges were responsible for <5% of the total water loss in three species of _Pogonomyrmex_ (Quinlan & Lighton 1999), a xeric adapted ant genus. Consequently, the permeability of the cuticle, and not respiratory water loss, has been measured in a variety of ants from xeric to mesic environments. CPs in insects range from 0.3 to 190 µg H₂O cm⁻²·mmHg⁻¹·h⁻¹ with those between 20 – 60 considered to be mesic and those under 20 µg H₂O cm⁻²·mmHg⁻¹·h⁻¹ considered to be xeric adapted (Edney 1977). Comparisons between various studies and species are more complicated because CP can be affected by caste variation or abrasion by soil (Johnson 2000), experimental conditions, and surface area calculations. When comparing CPs, we have attempted to adjust those values by calculating the surface area (SA) according to Haagsma et al. (1996), Lighton and Feener (1989) and Edney (1977).

The water loss data and response to temperature suggest that _L. luctuosum_ and _L. occidentale_ are intermediate in their responses compared with very xeric species such as _Pogonomyrmex_ or _Forelius_ and mesic species such as red imported fire ants and Argentine ants. Temper (1976) reported that _L. occidentale_ was intermediate in its responses to temperature and water loss compared with other California species. Temper (1976) found that workers of _L. occidentale_ lost 7.8 % of their body water at 2 h which is very similar to our findings. In their experiment, the xeric species _Messor_ (Veromessor) _anderi_ lost only 5.6% and the mesic species _Tapinoma sessile_ Say lost 12.9 % of its water, which means _L. occidentale_ lies within the middle of the water loss spectrum of California ants.
*Liometopum occidentale*, which were larger than *L. luctuosum* overall, had significantly more body water than *L. luctuosum*. This is consistent with the finding of Schilman et al. (2007) that larger species of ants had greater amounts of body water. The water loss rates were also higher for the smaller *L. luctuosum*, consistent with findings from Duncan and Lighton (1994) in which smaller workers of *Myrmecocystus mexicanus* Wesmael and *Myrmecocystus mendax* Wheeleri had greater water loss rates than larger workers. Interestingly the weights of two species of *Myrmecocystus* varied greatly with workers of *M. mexicanus* weighing between 1.6 to 36.8 mg and workers of *M. mendax* ranging from 1.6 to 11.6 mg whereas *L. occidentale* and *luctuosum* varied by only 2-3 mg, yet we observed the same effect of size. Similarly, Schilman et al. (2007) found that the larger workers of *S. xyloni* had significantly lower water-loss rates than did smaller workers. On the other hand, Appel et al. (1991) found that small *Solenopsis invicta* workers (0.53 mg) had significantly higher percentages of body water than did large workers (3.6 mg); however, the small workers had significantly higher CP (31.96 µg H$_2$O cm$^{-2}$mmHg$^{-1}$h$^{-1}$) than did the large workers (25.53 µg H$_2$O cm$^{-2}$mmHg$^{-1}$h$^{-1}$).

Large and small workers of both *L. luctuosum* and *occidentale* had similar CPs at both temperatures, ranging from 17.0 to 19.8 µg H$_2$O cm$^{-2}$mmHg$^{-1}$h$^{-1}$ when using the SA calculations determined by Haagsma (1996). When SAs were adjusted with the k = 0.103 (Schilman et al. 2007), the CP values of *L. luctuosum* and *occidentale* were only slightly higher than those calculated using the formula in Haagsma et al. (1996). CP values of a number of ant species were broadly associated with xeric and mesic environments as reviewed by Schilman et al. (2007) who used the formula from Lighton and Feener
Our values are higher than for the xeric species *Dorymyrmex insanus* (14.39), *Pogonomyrmex californicus* (Buckley) (13.99), *Crematogaster californica* (Wheeler) (6.13) and *Forelius mccooki* (McCook) (13.17) determined by Schilman et al. (2007) and the xeric desert harvester ant, *Messor pergandei* has a CP of 17 µg H₂O cm⁻² mmHg⁻¹ h⁻¹ (Lighton et al. 1994). Our CP values were intermediate between other xeric and mesic ant species such as red imported fire ants and Argentine ants.

Caution should be exercised when comparing CPs from different studies. Differences in CPs are greatly affected by the constants and formulas used to calculate surface area. The equation generated by Haagsma et al. (1996) works extremely well for insects less than 10 mg and variances were very small for both *L. luctuosum* and *occidentale*. The equation, \( SA = k[wt(g)]^{0.667} \) where \( k = 0.103 \) and \( wt \) equals insect weight in mg, generated by Lighton and Feener (1989) works very well for larger ants such as harvester ants which are closer to 10-20 mg and both equations provide very similar estimates of surface area. However, the equation by Lighton and Feener (1989) overestimates the surface area of smaller ants resulting in much higher CPs.

Further work is needed to determine the upper and lower temperature and humidity limits of *Liometopum*, as well as the effect of other abiotic factors. However, keep in mind that in order to accurately measure their cold tolerance, winter acclimated insects must be used. Rectal pad are, production of metabolic water, crop size, and behaviors exhibited under stress were all aspects of these ants that may explain their differences in survival but have yet to be explored.
REFERENCES


Table 3: Collection sites for *L. luctuosum* and *occidentale*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Colony #</th>
<th>Location info</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. occidentale</em></td>
<td>1</td>
<td>USA: CA: San Bernardino Co.: Crestline Lake Gregory Regional Park Elev. 1397m, 15 October 2011 34°14'28.94&quot;N 117°16'11.11&quot;W</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>USA: CA: San Bernardino Co.: Crestline Heart Rock near Camp Seeley Elev. 1280m, 3 August 2012 34°15'45.17&quot;N 117°18'16.56&quot;W</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>USA: CA: Riverside Co.: Riverside University of California- Riverside Elev. 321m, 19 August 2011 33°58’26.07”N 117°19’47.48”W</td>
</tr>
<tr>
<td><em>L. luctuosum</em></td>
<td>1</td>
<td>USA: CA, San Bernardino Co.: Crestline Lake Gregory Regional Park Elev. 1390m, 20 May 2012 34°14'25.62&quot;N 117°16'0.67&quot;W</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>USA: CA: San Bernardino Co.: Crestline Heart Rock near Camp Seeley Elev. 1280m, 3 August 2012 34°15'20.42&quot;N 117°18'19.50&quot;W</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>USA: CA: San Bernardino Co. Forest Falls Mill Creek near Forest Falls Picnic Area Elev. 1645m, 3 August 2012 34°05’3.21”N 116°53’51.68”W</td>
</tr>
</tbody>
</table>
Figure 10: Map of collection sites, in both San Bernardino Co. and Riverside Co.
Figure 11: Kaplan-Meier survival curves for *L. occidentale* and *L. luctuosum*

a) Temperature

Log-Rank Test: $\chi^2 = 1038.804$

DF = 2, $P < 0.001$
b) Humidity

Log-Rank Test: $\chi^2 = 57.655$

$DF = 2, P < 0.001$
c) Species

Log-Rank Test: $\chi^2 = 141.592$

DF = 2, $P < 0.001$
d) Saturation Deficit

Log-Rank Test: $\chi^2 = 1326.850$
$DF = 8, P < 0.001$
Figure 12: Kaplan-Meier survival curves for *L. luctuosum* colonies

Log-Rank Test: $\chi^2 = 141.592$
DF $= 2$, $P < 0.001$
Figure 13: Kaplan-Meier survival curves for *L. occidentale* colonies

Log-Rank Test: $\chi^2 = 111.118$

DF = 2, $P < 0.001$
Table 4: Multiple comparisons of model factors for the model: $Time*Dead(0) = Temp + Hum + Specolony + Temp*Hum$ with both species included in the model.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>Alpha</th>
<th>Confidence</th>
<th>Chi-Square</th>
<th>Pr&gt;ChiSq</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1-T2</td>
<td>0.1449</td>
<td>0.0141</td>
<td>0.05</td>
<td>0.1198</td>
<td>394.4895</td>
<td>&lt;.0001</td>
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<tr>
<td>T1-T3</td>
<td>0.0089</td>
<td>0.00129</td>
<td>0.05</td>
<td>0.00669</td>
<td>1054.4327</td>
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<tr>
<td>T2-T3</td>
<td>0.0614</td>
<td>0.00684</td>
<td>0.05</td>
<td>0.0494</td>
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</tr>
<tr>
<td>H1-H2</td>
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<td>0.1996</td>
<td>0.05</td>
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<td>131.3502</td>
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<tr>
<td>H1-H3</td>
<td>4.2858</td>
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<td>3.6096</td>
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<tr>
<td>H2-H3</td>
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<td>0.1445</td>
<td>0.05</td>
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<td>S1C1-C2</td>
<td>0.9469</td>
<td>0.1021</td>
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<td>0.2561</td>
<td>0.6128</td>
</tr>
<tr>
<td>S1C1-C3</td>
<td>5.847</td>
<td>0.6733</td>
<td>0.05</td>
<td>4.6656</td>
<td>235.1456</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>S1C2-C3</td>
<td>6.1749</td>
<td>0.7165</td>
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<td>4.9188</td>
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<tr>
<td>S2C1-C2</td>
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<td>0.2209</td>
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<td>27.8736</td>
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<tr>
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<td>0.2817</td>
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<td>1.9280</td>
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Table 5: Multiple comparisons of model factors for the model: $Time*Dead(0) = Temp + Hum + Colony + Temp*Hum$ (Likelihood Ratio $\chi^2 = 903.9162$, df= 10, p-value < 0.0001) for *L. luctuosum*

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>Alpha</th>
<th>Confidence Limits</th>
<th>Chi-Square</th>
<th>Pr&gt;ChiSq</th>
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<td>0.000739</td>
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<td>0.00184, 0.00485</td>
<td>553.209</td>
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<tr>
<td>T2-T3</td>
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<td>0.00527</td>
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<tr>
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<td>3.0543, 5.0365</td>
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<td>0.05</td>
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<td>162.2537</td>
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<td>0.05</td>
<td>1.1831, 2.0585</td>
<td>9.9217</td>
<td>0.0016</td>
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<td>C1-C3</td>
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<td>0.1814, 1.4455</td>
<td>1.5981</td>
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Table 6: Multiple comparisons of model factors for the model: $Time*Dead(0) = Temp + Hum + Colony + Temp*Hum$ (Likelihood Ratio $\chi^2 = 718.4493$, df= 10, p-value < 0.0001) for *L. occidentale*

<table>
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<tr>
<th>Contrast</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>Alpha</th>
<th>Confidence Limits</th>
<th>Wald Chi-Square</th>
<th>Pr &gt; ChiSq</th>
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<tr>
<td>T1-T2</td>
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<td>0.0233</td>
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<td>0.1303 0.2227</td>
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<tr>
<td>T1-T3</td>
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<td>0.00324</td>
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<td>0.0115 0.0245</td>
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<td>T2-T3</td>
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<td>0.05</td>
<td>0.0731 0.1322</td>
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<td>H1-H2</td>
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<td>H1-H3</td>
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<td>1.4482 2.2336</td>
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<tr>
<td>C1-C3</td>
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<td>4.3322 7.0893</td>
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<tr>
<td>C1-C2</td>
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<td>0.7952 1.2176</td>
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<td>C2-C3</td>
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<tr>
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<td>0.5986 1.6772</td>
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<td>0.7968 4.6497</td>
<td>2.1175</td>
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Table 7: Multiple comparison of the following model: $\text{Time} \cdot \text{Dead}(0) = \text{SD} + \text{Specolony}$ (Likelihood Ratio $\chi^2 = 1715.5165$, df= 13, p-value < 0.0001; Specolony= Species(Colony)) for both species included in the model.

<table>
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<tr>
<th>Contrast</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>Alpha</th>
<th>Confidence Limits</th>
<th>Wald Chi-Sq</th>
<th>Pr&gt;ChiSq</th>
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<tbody>
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<td>SD8.7-SD6</td>
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<td>0.0533</td>
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<td>SD8.7-SD3.6</td>
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<td>0.3227</td>
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<tr>
<td>SD8.7-SD15</td>
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<td>0.0963</td>
<td>23.6020</td>
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<td>0.00181</td>
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<td>0.00099</td>
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<tr>
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<td>0.05</td>
<td>4.8524</td>
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<tr>
<td>SD6-SD15</td>
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<td>0.05</td>
<td>1.6833</td>
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<tr>
<td>SD6-SD10.75</td>
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<td>SD6-SD5.6</td>
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<td>SD6-SD17</td>
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<tr>
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<td>0.0575</td>
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<td>0.7165</td>
<td>0.05</td>
<td>4.9188</td>
<td>7.7518</td>
<td>246.1469</td>
</tr>
<tr>
<td>S2C1-C2</td>
<td>1.8673</td>
<td>0.2209</td>
<td>0.05</td>
<td>1.4809</td>
<td>2.3545</td>
<td>27.8736</td>
</tr>
<tr>
<td>S2C1-C3</td>
<td>2.4216</td>
<td>0.2817</td>
<td>0.05</td>
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<td>3.0417</td>
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<tr>
<td>S2C2-C3</td>
<td>1.2969</td>
<td>0.148</td>
<td>0.05</td>
<td>1.0369</td>
<td>1.622</td>
<td>5.1866</td>
</tr>
<tr>
<td>S1-S2</td>
<td>214.6</td>
<td>49.4343</td>
<td>0.05</td>
<td>136.6</td>
<td>337</td>
<td>543.0314</td>
</tr>
</tbody>
</table>
Table 8: Cuticular permeability and percent body water content of workers of *L. occidentale* and *luctuosum*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Temp. (°C)</th>
<th>N</th>
<th>Body water ratio</th>
<th>Body mass range (mg)</th>
<th>CP’s(^b)</th>
<th>CP’s(^c)</th>
<th>CP’s(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. occidentale</em></td>
<td>25.3</td>
<td>20</td>
<td>0.70 ± 0.017</td>
<td>1.44-5.24</td>
<td>17.0 ± 6.46</td>
<td>17.8 ± 7.13</td>
<td>15.3 ± 6.13</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>20</td>
<td>0.67 ± 0.033</td>
<td></td>
<td>19.8 ± 4.82</td>
<td>20.5 ± 4.68</td>
<td>27.9 ± 6.38</td>
</tr>
<tr>
<td><em>L. luctuosum</em></td>
<td>25.3</td>
<td>18</td>
<td>0.65 ± 0.038</td>
<td>0.91-3.53</td>
<td>17.8 ± 7.08</td>
<td>21.7 ± 9.01</td>
<td>18.6 ± 7.75</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>20</td>
<td>0.62 ± 0.039</td>
<td></td>
<td>19.1 ± 4.76</td>
<td>26.8 ± 8.65</td>
<td>36.6 ± 11.8</td>
</tr>
</tbody>
</table>

\(^b\) SA = Surface area (cm\(^2\)) = 0.09 + 26.85[wt(g)] – 214.21[wt(g)]\(^2\) (Haagsma et al. 1996)

\(^c\) SA = 0.103 [wt (g)]\(^{0.667}\) (Lighton and Feener 1989)

\(^d\) SA = k [wt (g)]\(^{0.667}\) (Edney 1977, Appel et al. 1983, 1991)
Figure 14: Distribution of worker weights (mg) of *L. luctuosum* and *L. occidentale*.
Figure 15: Water loss rates of *L. occidentale* and *luctuosum*. 
Chapter 4:

Interspecific Interactions and Population genetics of

Liometopum occidentale
ABSTRACT

Liometopum occidentale appear to nest within the roots of oak trees or under larger boulders making them inaccessible and extremely difficult to examine determine nest structure and queen number. A segment of the mitochondrial (mtDNA) COI gene was sequenced and used to determine that L. occidentale nest contain either one queen or multiple maternally related queens. Observations of the behaviors displayed by workers separated in an urban landscape were made and used to determine that there is a slight but significant correlation between geographic distance and their level of aggression.

INTRODUCTION

Ants belonging to the genus Liometopum are regionally distributed across North America, Europe and Asia. Liometopum apiculatum Mayr, L. luctuosum Wheeler, and L. occidentale Emery are found in western North America and are referred to as velvety tree ants. Most species are associated with trees, but in recent years both L. luctuosum and L. occidentale have been reported as urban pests causing structural damage (Wheeler & Wheeler 1986, Merickel & Clark 1994, Gulmahamad 1995, Hedges 1998, Klotz et al. 2008). Their importance as structural pests is really unknown because these ants are frequently misidentified as carpenter ants and there has also been increased urbanization of rural and mountain areas of the western U.S. Very little is known about the biology of these species.

Colonies have been estimated to contain between 40,000 and 60,000 workers (Ramos-Elorduy & Levieux 1992, Del Toro et al. 2009). It is uncertain whether all these
ants are unicolonial or whether there are many colonies with distinct boundaries. Wang et al. (2010) examined the nest mate recognition of 18 colonies of *L. occidentale* in James Reserve and Stunt Ranch in southern California. They observed greater levels of aggression between workers from the different locations relative to the levels observed within each location; but, they also observed that the level aggression was not completely uniform across pairings. Nevertheless, they concluded that *L. occidentale* workers did not universally accept each other. Results of this study also showed that ants from sites separated by more than a kilometer were not aggressive toward one another. Wang et al. (2010) concluded that these ants may be unicolonial within a location. They also observed ants from some locations over 100 km apart were also not aggressive to one another. In this case they speculated that they shared similar genetic markers, in which case, there would be few recognition ‘alleles’ that distinguish colony members throughout the range of this species. Another possibility was that recognition was predominantly determined through environmental cues that are locally shared, such as type nesting material, habitat type, etc. After finding no aggression between workers collected from significant distances apart and no territory boundary, Wang et al. (2010) speculated that *L. occidentale* colonies are large and polydomous. Since they never found brood or queens, it is uncertain whether there are multiple queens within a nest, or whether each queen has some localized “sphere of exclusivity.” It seems unlikely that there could be just one queen that produces enough eggs to make a colony that is one kilometer wide, so they speculated that *L. occidentale* are polygyne.
Due to the inaccessibility of _L. occidentale_ nests, it is difficult or impossible to collect and examine entire colonies and to determine the number of queens within them. Stille and Stille (1992) found that analysis of mitochondrial restriction fragment length polymorphisms (mtDNA RFLP) is a method well suited for investigations of the population structure of ants. Also, the use of selectively neutral loci, has established that reductions in genetic diversity, reflecting founder effects, have occurred during the establishment of some invasive populations. Some colonial organisms may actually gain an ecological advantage from reduced genetic diversity because of the associated reduction in inter-colony conflict (Smith et al. 2012). Sequencing of a segment of mitochondrial (mtDNA) cytochrome oxidase c subunit 1 (COI) may be sufficient to gain a basic understanding whether a species is native or invasive. This method has been used for the colonial sea squirt, _Didemnum vexillum_ (Smith et al. 2012).

One of the objectives was to sequence the mitochondrial (mtDNA) COI gene to try to determine whether these ants were truly native and polygynous. We also observed how colonies of these ants separated in an urban landscape interact and whether there was a correlation between their level of aggression and geographic distance. Furthermore, we determined how these ants interact with other native ant species, _Dorymyrmex bicolor_ Wheeler and _Formica fusca_ grp, and the invasive species, _Linepithema humile_ and _Solenopsis invicta_ Buren.
MATERIALS AND METHODS

Ant collections

*Liometopum occidentale* foragers were collected from 18 sites (2 from the same location but different years; site 19; Fig. 16, 17 and Table 9) in Riverside and Crestline, CA. Ants were collected in plastic boxes coated on the inside with Teflon T-30B (Dupont) to prevent them from escaping. Ants were collected by gently brushing foragers off the trunk of the trees using a soft bristled paint brush into the plastic boxes. Live workers were taken to the University of California, Riverside, where they were maintained in the laboratory in the boxes they were collected in and provided harborage consisting of a Petri dish (14 cm diameter) which was half filled with plaster of Paris, as well as a continuous supply of water, 25% sucrose water, and pieces of dead American cockroaches, *Periplaneta americana* L. All species were maintained within the lab for at least a week before use in this experiment. Ants for genetic study were collected on site and stored in vials of absolute ethanol (100%) at 32°C.

Genetic evaluation of relatedness and polygyne

The polymerase chain reaction (PCR) was used to amplify a section of the mitochondrial (mtDNA) cytochrome oxidase c subunit 1 (COI) gene of 5-10 workers per collection site. PCR was performed in 25 µL reactions containing 2 µL of DNA template (concentration not determined), 1X ThermoPol PCR Buffer (New England BioLabs, Ipswich, MA), an additional 1 mM MgCl₂, 200 µM dATP, 200 µM dCTP, 200 µM dGTP, 400 µM dUTP, 4% (v/v) BSA (NEB), 1 U Taq polymerase (NEB), and 0.2 µM
each of the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3’) (Folmer et al. 1994).

Thermocycling was performed in a Mastercycler® ep gradient S thermocycler (Eppendorf North America Inc., New York, NY) programmed for an initial denaturing step of 2 min at 94 °C; followed by five cycles of 30 s at 94 °C, 1 min 30 s at 45 °C, and 1 min at 72 °C; followed by a further 35 cycles of 30 s at 94 °C, 1 min 30 s at 51 °C, and 1 min at 72 °C; and a final extension of 5 min at 72 °C. Amplification was confirmed by gel electrophoresis and PCR products were subsequently cleaned using the Wizard® PCR Preps DNA purification system (Promega, Madison, WI) and direct-sequenced in both directions at the University of California Riverside Genomics Institute, Core Instrumentation Facility. This was a standard procedure used by Rugman-Jones et al. (2012).

All sequences were trimmed and aligned manually in BioEdit 7.0.5.3 (Hall 1999). The alignment of COI sequences resulted in a matrix of 116 sequences, each approximately 660 base pairs in length. The absence of nuclear pseudogenes (Song et al. 2008) was confirmed by translating the COI sequences using the Transeq application in EMBOSS (http://www.ebi.ac.uk/Tools/emboss/transeq/index.html), and representative sequences will be deposited in GenBank® (Benson et al. 2008). Sequence polymorphism, within the dataset as a whole, was assessed as number of haplotypes. TCS v1.21 was used to construct a simple haplotype network.
Aggression assay

Fifty or more *L. occidentale* workers from each collection site (“colony”) were separated into small containers, anesthetized with CO\(_2\), and a dot of paint placed on the dorsal surface of their gaster with oil based paint from a Sharpie brand or Craft Smart\(^{®}\) brand paint pen. An area design such as Fig 19 was used for these assays. Five ants from one colony were placed within the smaller tube which was coated with Teflon T-30B (E.I. du Dupont Nemours and Co., Wilmington, DE) on both sides and was then inverted within the large dish which was coated with Teflon T-30B on the inner sides, then five ants from the other colony were placed in the large dish outside the smaller tube. Ants were allowed to acclimate in the arena for 30 min and then the tube was removed allowing the ants to move freely about the arena and encounter one another. Ants in the arenas were video recorded for 5 min, using a Canon ZR 960 miniDV Digital Video Camcorder. Videos were reviewed and the first 15 interactions between ants from different colonies were rated on a scale of 0 to 7: 0-ignore, 1-move away slowly, 2-touch, 3-move away quickly (“avoid), 4-aggressive display, 5-nip at or bite onto opponent, 6-“lock-and-roll”, 7-dismember. Colony pairings (n = 36) were chosen to optimize the number of distance categories and haplotypes compared (Table 10). Six to ten replicates were conducted for each pairing.

Statistical Analyses

Pearson Chi-square (SYSTAT13) and Kruskal-Wallis (SYSTAT13) tests were used to determine whether there was an overall difference in the frequency distributions
of behaviors (level of aggression) displayed by *L. occidentale* workers toward each other based on distance between them (Null Hypothesis: Behavior is independent of distance). Tukey-HSD style multiple comparisons (Winks 7.05 Basic Edition) were used to separate the distance categories based on the behavioral frequency distributions (Zar 2010). Pearson Chi-squared and Kruskal-Wallis were used to determine whether there was an overall difference in the behavior frequency distributions (level of aggression) of colonies within the same distance categories. Spearman’s Rho was used to determine the correlation between distance and behavior frequency (level of aggression) (SYSTAT13).

**RESULTS**

**Intraspecific interactions**

*Aggressive Behavior by distance*

As the distance increased between different colonies of *L. occidentale*, the level of aggression between them increased. When behavioral interactions were compared to the distance between colonies using three distance categories (1: <1km, 2: 1.0-6.0km, 3: >8.0km) all three distances were significantly different from each other (Figure 21; \( \chi^2 = 291.697, \text{df}= 12, P< 0.0001; 1 \text{ vs. } 2: \chi^2 = 65.849, \text{df}= 6, P<0.0001; 1 \text{ vs. } 3: \chi^2 = 228.156 \text{ df}= 6, P< 0.0001; 2 \text{ vs. } 3: \chi^2 = 123.587 \text{ df}= 6, P< 0.0001\). Distance categories can be ordered from lowest to highest aggression (rank sum), as follows: 1 < 2 < 3 (H= 107.930, \text{df}= 2, P< 0.0001). The proportions of aggressive behaviors 4, 5, and 6 increased with distance. In fact, the proportion of behaviors that were behavior 4 nearly doubled from distance category 1 to 2, and increased by one-third from distance category 2 to 3 (Figure...
The proportion of behaviors that were categorized as level 5 did not increase from distance category 1 to 2, but doubled from category 2 to 3 (Figure 21). The proportion of behaviors that were behavior 6 did not increase from distance category 1 to 2, but was six times higher from distance category 2 to 3 (Figure 21). When the behaviors were pooled into two categories, not aggressive or aggressive, the level of aggressive of each distance category was significantly different, and could still be ordered from lowest to highest aggression (rank sum) as follows: 1 < 2 < 3 (H= 209.23, df=2, P< 0.001). When the behaviors were pooled into three categories, not aggressive, passive, or aggressive, the level of aggressive of each distance category was significantly different, and could still be ordered from lowest to highest aggression (rank sum) as follows: 1 < 2 < 3 (H= 182.58, df=2, P< 0.001).

When 5 distance categories (1: <0.5km, 2: 0.5-2.0km, 3: 4.0-6.0km, 4: 8.0-12.0km, 5: >16.0km) were compared, distances were significantly different (Figure 22; \( \chi^2 = 366.20515 \) df= 24 P< 0.0001). Distance categories can be ordered from lowest level of aggression to highest (based on rank sums), as follows: 1 ≤ 2 < 3 ≤ 5 ≤ 4 (H= 112.42452, df= 4, P< 0.0001). The frequency of behaviors 4, 5, and 6 increased as the distance between colonies increased. In fact, the proportion of behaviors that were category 4 more than doubled from distance category 1 to 2, increased by one-third from distance category 2 to 3 and from 3 to 4, but decreased from category 4 to 5 (Figure 22). The proportion of behaviors that were behavior 5 did not increase from distance category 1 to 2 and 4 to 5, but increased from category 2 to 3 and nearly doubled from category 3 to 4 (Figure 22). The proportion of behaviors that were behavior 6 did not increase from
distance category 1 to 2 and 4 to 5, but increased from category 2 to 3 and more than quadrupled from distance category 3 to 4 (Figure 22). When the behaviors were pooled into two categories, not aggressive or aggressive, the level of aggression of each distance category could still be ordered from lowest to highest aggression (rank sum) as follows: 1 ≤ 2 < 3 < 5 ≤ 4 (H= 232.6, df=2, P< 0.001). When the behaviors were pooled into three categories, not aggressive, passive or aggressive, the level of aggressive of each distance category could still be ordered from lowest to highest aggression (rank sum) as follows: 1 < 2 ≤ 3 < 5 < 4 (H= 206.1, df=2, P< 0.001).

Behavior of colonies within distance categories

The three distance categories were further analyzed since they separated clearly in tests. The behaviors displayed by *L. occidentale* workers varied by colony within the three distance categories (Distance 1: $\chi^2 = 306.4$, df= 22, P< 0.0001; Distance 2: $\chi^2 = 253.13834$, df= 18, P< 0.0001; Distance 3: $\chi^2 = 190.46577$ df= 20, P< 0.0001). This means that some colonies were more aggressive than others with some pairing being more incompatible.

**Correlation between distance and behavior/aggression**

There was a slight but significant positive correlation of distance and level of aggression (Spearman Rho correlation coefficient: 0.09974, P < 0.0001). In other words as distance increased, the frequency of higher behavioral scores (4, 5, 6) or the level of aggression also increased.
Genetic analysis of mtDNA

The 17 sites (plus two sets from site 19 but different years) examined contained a total of 8 different sequences (haplotypes) of mitochondrial (mtDNA) cytochrome oxidase c subunit 1 (COI) (Table 10). Figure 20 represents the relationship between the 8 different haplotypes and the number of base pair differences between them.

**DISCUSSION**

Mitochondrial DNA

Eight sequences (haplotypes) of mitochondrial (mtDNA) cytochrome oxidase c subunit 1 (COI) from 17 sites is a high level of variability. This high level of variation suggests that these ants did not go through a population bottleneck and therefore must be native. Since COI is just a single maternally inherited gene, each of the collection sites (“colonies”) is most likely built around a single queen, or perhaps a handful of queens that are derived from an initial single queen. However, it reveals nothing about levels of colony mixing. Each individual queen could potentially have mated with one or more males from different genetic backgrounds (colonies). To answer this question it would require looking at nuclear loci.

Restriction fragment length polymorphisms (RFLPs) of mitochondrial DNA of other ant species, such as *S. invicta* (Shoemaker et al. 2006) and *Iridomyrmex purpureus* (Smith) (Carew et al. 1997) have used to determine number of queens within a nest and relatedness of queens. Variation in the mitochondrial DNA also enables the detection of
past entry of external queen lineages into the colony, such as has been shown for


The number of differences between the different haplotypes is in itself a measure of genetic distance, if we assume a stepwise model of sequence evolution. If this is so, then each change/mutation is an individual evolutionary event, so haplotypes that are 2bp different are more genetically distant than those that are only 1bp. However, genetic distance still says nothing definitive about relatedness at the colony level.

RFLPs, RAPDs, AFLPs, microsatellites, and SNPs could all potentially be developed and used in future studies to address questions of colony and population level genetics.

**Aggression**

Animals assess many factors when choosing how aggressively to behave during interspecific interactions (Tanner & Alder 2009). Although aggressive behavior towards competitors is sometimes necessary to win resources, it can be costly in terms of energy spent, time lost, and possible injury or mortality (Jaeger 1981; Cole 1986; Tanner & Alder 2009). Behaviorally dominant ant species commonly use a strategy in which they aggressively defend the nest but only reciprocate the behaviors of their opponents when encountering them away from the nest (Tit-for-Tat strategy). While on the other hand, more submissive ant species usually favor a highly context-dependent strategy in which they only aggress near their most valuable resource, usually their nest (Tanner & Alder 2009).
The optimal level of aggression displayed by an individual towards a competitor can vary with the context of the interaction, examples include: seasonal variation and/or reproductive cycle (Thurin & Aron 2008), food availability (Downs & Ratnieks 2000), familiarity between competitors (Ydenberg et al. 1988; Temeles 1994), resource value (Reeve 1989), habitat type (Rodriguez 1995; Langkilde & Shine 2004), disparity between contestants’ fighting abilities (Maynard Smith 1979; Riechert 1998), and the competitor’s behavior during the contest (Riechert 1998).

Researchers have used a variety of methods to test aggression and nest mate recognition in ants. Small numbers of ants have been tested against each other or group interactions have been observed. Detailed observations or simple counts have been made of individuals fighting/dead. Common bioassays include individual live-on-dead interactions, individual live on live interactions, group (5+) live on live interactions, and colony merging. According to Roulston et al. (2003), assays that utilized the greatest number of live ants were the most likely to reveal high levels of aggression. Live group interactions and colony introduction assays are most consistent across replicates. Ranking scales for the variety of behaviors ants exhibit when encountering another individual can be created to assess aggression. These scales can include every behavior possible or just key behaviors. The designs of test arenas vary as well. Roulston et al. (2003) employed a design that consisted of a plastic ring or dish with a fluon-coated wall and a smaller ring within this larger ring creating two separate arenas until the small ring is removed. Fénéron (1996) used a more complex design in which petri-dishes were divided into quadrants and a fixation system that was used to immobilize adult ants. The first design
allows both individuals to interact as they would in nature; however, individuals in this case can only be used tested once, as they could be injured during the encounter. The second design allows the unrestrained individual to be used for multiple replicates. Recording time intervals vary as well from minutes (small number of encounters) to days (large number of encounters usually using whole or subsets of colonies).

To tease apart the effect of geography on the level of aggression of *L. occidentale* we chose to use a scale of behaviors with a range from non-aggressive behaviors to highly aggressive behaviors in which the least aggressive behaviors began in the middle categories. I also chose to use 5-on-5 live ant assays because this allowed more interactions.

*Liometopum* are generally described as highly competitive, behaviorally dominant ants. The North American species, *L. occidentale, L. apiculatum* and *L. luctuosum* are said to play an ecologically similar role to the behaviorally dominant Australian dolichoderines, *Anonychomyrma, Papyrius* and *Froggattella* (Andersen 1997). The interactions between *Liometopum* spp. and native ants have been studied for *L. microcephalum* Panzer, an Old World species. *L. microcephalum* can be very aggressive toward other ant species, attacking by biting and spraying secretions that repels enemies, and initiate alarm behavior (Petráková & Schlaghamerský 2011). Aggressive behavior occurs close to the nest, on trails, on trees, and occasionally at food resources. *L. microcephalum* takes advantage of worker cooperation during aggressive interactions, a strategy used by smaller ants (Petráková & Schlaghamerský 2011).
Over 50% of the behaviors displayed by *L. occidentale* workers toward workers of the same species but from other colonies were non-aggressive categories 0, 1 or 2. This resulted in behavior frequency distributions skewed strongly toward the non-aggressive behaviors. Some possible explanations for this low level of aggression are there may only be a few recognition alleles within the range of this ant species, there is likely some effect of the degree of relatedness within a colony and through the range of this ant, and environmental factors such as diet and nest material. Wang *et al.* 2010 speculated that the low level of aggression in *L. occidentale* may be because these ants have relatively few recognition alleles that distinguish colony members throughout the range of this species. A similar mechanism is proposed for variation in aggression within and across two *Pheidole* species (Langen *et al.* 2000; Tripet *et al.* 2006).

Another possible explanation for these levels of aggression is the relatedness of individuals. If relatedness within a colony is low, this would result in many recognition cues within a colony. Low relatedness within a colony could result from multiple mating of queens or multiple queens within the nest. However, if relatedness is high across the range of this species, it is more beneficial for colonies to not aggressively respond to one another. High relatedness of individuals across the range of a species can result from many factors, such as inbreeding, and colony reproduction by budding. The genetic relatedness between colonies of this species still remains to be studied.

Environmental conditions, such as nesting material or diet, have been found to have an affect both colony odor and nestmate discrimination. For example *Temnothorax nylanderi* (Foerster) workers react more aggressively to alien ants from nests in other
types of wood than to non-nestmates living in the same nesting material (Heinze et al. 1996). Workers were more aggressive to former nestmates when colonies were experimentally split and the fragments were housed in different nesting material than when the fragments nested in the same type of wood (Heinze et al. 1996). Diet can also have an affect the nestmate recognition behavior and level of aggression of a number of ant species including, *Acromyrmex octospinosus* (Reich) (Jutsum et al. 1979), *S. invicta* (Obin & Vander Meer 1988), *Formica cunicularia* Latreille (Le Moli et al. 1992), *L. humile* (Liang & Silverman 2000, Chen & Nonacs 2000), *Acromyrmex subterraneus* Forel (Richard et al. 2004); however, in other species such as *Odontomachus bauri* (Jaffê & Marcuse 1983) it has no effect. Even geographical variations in the effect of the diet on cue expression and/or perception by other individuals were reported in the *L. humile* (Buczkowski & Silverman 2006). However, these environmental factors are more likely to be similar at shorter distances, resulting in similar colony odors within colonies closer to each other and different odors within colonies from sites in different habitats. This would result in the pattern of higher aggression between colonies separated by further distances that we observed in this species.

The aggressive behavior of these ants could also be due to seasonal effects. Thurin and Aron (2008) found that there were significant seasonal variations in the levels of aggression among workers of different colonies of the ant, *Plagiolepis pygmaea* (Latreille), and that this was probably attributed to the biological cycles of the species. In this species, aggressiveness was higher in spring and lower in the summer and autumn. This finding coincided with the availability of food and the reproductive cycle of the
colony. Reproductives of *L. occidentale* have been observed flying in May (Del Toro *et al.* 2009, Wang *et al.* 2010). Competition between these ant colonies with other ants for food resources would be greatest during this time when they are collecting food for the developing reproductive brood.

We found that the frequency of higher level aggressive behaviors *L. occidentale* workers exhibited increased with increased distance between colonies. Some possible explanations for these results are that competitors separated by shorter distances were more likely to be familiar with each other or at least some of the recognition cues they contain (“dear enemy” effect); relatedness likely played a role in determining these results as well, such as the likelihood that relatedness increased with decreased spatial distance; environmental factors such as diet and habitat could have played a role as well.

Competitor familiarity can have an unpredictable effect on level of aggressive expressed between competing individuals. Evidence suggests that, across taxa, familiarity can either increase (Müller and Manser 2007; Thomas *et al.* 2007) or decrease (Fisher 1954; Jaeger 1981; Gordon 1989; Langen *et al.* 2000) aggression between competitors (examples of both cases reviewed in Ydenberg *et al.* 1988; Temeles 1994). In our case the positive correlation of spatial distance and level of aggression, may be explained by the “dear enemy” effect, which is an ethological phenomenon in which a territorial animal responds more strongly to strangers than it does to neighbors from adjacent territories. This phenomenon may be generally advantageous to an animal because it minimizes time and energy spent on territorial defense, and reduces the risk of injury during territorial encounters. Examples of other ant species that also display this
phenomenon include: *Pheidole tucsonica* and *P. gilvescens* Creighton and Cregg (Langen et al. 2000). Langen et al. (2000) showed that neighbors (i.e., colonies less than 2.6 m away) of either species are treated less aggressively than more distant colonies and that habituation may be a mechanism by which this discrimination is achieved.

The aggressive behavior of *L. occidentale* workers from different colonies separated by the same distance varied. Some possible explanations for these results are that the type of aggression these ants use during competitive interactions, the disparity between contestants’ fighting ability, etc. The behavior of a competitor often has a significant effect on the behavior of the contestant (Riechert 1998). Competitor behavior can elicit more aggressive behaviors from an individual, such as for individuals that use the Tit-for-Tat strategy in which the contestant initially cooperates with the competitor but then reciprocates the last action of its opponent during subsequent interactions. Competitor behavior can also have contradictory effects on an individual’s behavior, such as the display of aggressive behaviors by an aggressive competitor which elicits a nonaggressive escape response from the contestant (Schelling 1960). There can also be disparity between contestants fighting ability, resulting in variability of the level of aggression between colonies. For example, this has phenomenon has been described by Maynard Smith (1979) and Riechert (1998) in reviews of game theory and animal behavior.

Further work is needed to determine the effect of factors such as diet and nest material. This could be achieved by separating colonies and feeding them different diets and/or giving them different types of nesting material. The effect of separation time and
possible changes in the hydrocarbon profiles on level of aggression would be another interesting aspect of nestmate recognition behavior of *L. occidentale* to explore. Again this could be achieved by separating colonies for variable amounts of time and measuring their level of aggression toward each other. Since seasonal differences of colony development have been observed in these ants, seasonal effects on levels of aggression should be explored as well. The relatedness of neighboring colonies should also be determined to further explain relationships of aggression level and distance.
REFERENCES


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SYSTAT 13. 2009. SYSTAT Software Inc., San Jose, CA.


Figure 16: Map of collection sites in USA: Riverside Co.: Riverside, CA
Figure 17: Map of collection sites in San Bernardino Mountains, San Bernardino Co., CA
Figure 18: All collection sites in Riverside Co. and San Bernardino Co.
Table 9: Location information each collection site

<table>
<thead>
<tr>
<th>Site Number</th>
<th>Location info</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>USA: CA, Riverside Co., Riverside University of California-Riverside, Ag Ops 33°57'50.50&quot;N 117°20'49.81&quot;W, 295.35m Eucalyptus</td>
</tr>
<tr>
<td>2</td>
<td>USA: CA, Riverside Co., Riverside University of California-Riverside 33°58'26.07&quot;N 117°19'47.48&quot;W, 320.65m Oak-grass</td>
</tr>
<tr>
<td>3</td>
<td>USA: CA, Riverside Co., Riverside University of California-Riverside, Director's Garden 33°58'02.54&quot;N 1147°19'33.52&quot;, 356.01m Oak-Grass</td>
</tr>
<tr>
<td>4</td>
<td>USA: CA, Riverside Co., Riverside University of California-Riverside, Health Center 33°58'33.86&quot;N 117°19'30.78&quot;W, 327.66m Olive tree</td>
</tr>
<tr>
<td>5</td>
<td>USA: CA, Riverside Co., Riverside University of California-Riverside, Health Center bridge 33°58'37.71&quot;N 117°19'35.88&quot;W, 324m Oak</td>
</tr>
<tr>
<td>6</td>
<td>USA: CA, Riverside Co., Riverside University of California-Riverside, Belltower 33°58'24.62&quot;N 117°19'43.71&quot;W, 322.48m Oak-grass</td>
</tr>
<tr>
<td>7</td>
<td>USA: CA, Riverside Co., Riverside Fairmount Park Lake #1 33°59'49.17&quot;N 117°22'34.59&quot;W, 243.23m Oak</td>
</tr>
<tr>
<td>8</td>
<td>USA: CA, Riverside Co., Riverside Fairmount Park Lake #2 33°59'53.33&quot;N 117°22'45.73&quot;W, 240.49m Oak</td>
</tr>
<tr>
<td>9</td>
<td>USA: CA, Riverside Co., Riverside</td>
</tr>
<tr>
<td>No.</td>
<td>Location</td>
</tr>
<tr>
<td>-----</td>
<td>----------</td>
</tr>
<tr>
<td>11</td>
<td>USA: CA, Riverside Co., Riverside Fairmount Park, Lrg Playground</td>
</tr>
<tr>
<td>12</td>
<td>USA: CA, Riverside Co. Rancho Jurupa Regional Park, Primitive camp site</td>
</tr>
<tr>
<td>13</td>
<td>USA: CA, Riverside Co., Riverside Rancho Jurupa Regional Park, Hiking Trail</td>
</tr>
<tr>
<td>15</td>
<td>USA: CA, Riverside Co., Riverside Rubidoux Nature Center</td>
</tr>
<tr>
<td>16</td>
<td>USA: CA, Riverside Co. Riverside, CA- Rubidoux Nature Center</td>
</tr>
<tr>
<td>17</td>
<td>USA: CA, Riverside Co., Riverside Hidden Valley Nature Center</td>
</tr>
<tr>
<td>18</td>
<td>USA: CA, Riverside Co., Riverside Hidden Valley Nature Center, Horse Park</td>
</tr>
<tr>
<td>19</td>
<td>USA: CA, Riverside Co., Crestline Lake Gregory Regional Park</td>
</tr>
</tbody>
</table>
Figure 19: Aggression assay arena design
Table 10: Colonies tested, their haplotypes, distance categories, etc.

<table>
<thead>
<tr>
<th>Colonies</th>
<th>Haplotypes</th>
<th>No. of diffs in haplotype</th>
<th>Distance (km)</th>
<th>Distance Catagory (km)</th>
<th>Distance Catagory (km)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15/16</td>
<td>C/E</td>
<td>6</td>
<td>0.0616</td>
<td>&lt;1</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>2/6</td>
<td>B/B</td>
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<td>0.1570</td>
<td>&lt;1</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>12/13</td>
<td>D/C</td>
<td>3</td>
<td>0.2167</td>
<td>&lt;1</td>
<td>&lt;0.5</td>
</tr>
<tr>
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<td>A/G</td>
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<td>0.2267</td>
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<td>&lt;0.5</td>
</tr>
<tr>
<td>7/8</td>
<td>G/G</td>
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<td>0.3131</td>
<td>&lt;1</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>17/18</td>
<td>A/A</td>
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<td>0.4415</td>
<td>&lt;1</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>2/5</td>
<td>B/A</td>
<td>2</td>
<td>0.5003</td>
<td>&lt;1</td>
<td>0.5-2.0</td>
</tr>
<tr>
<td>2/4</td>
<td>B/C</td>
<td>7</td>
<td>0.5605</td>
<td>&lt;1</td>
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</tr>
<tr>
<td>2/3</td>
<td>B/F</td>
<td>8</td>
<td>0.8214</td>
<td>&lt;1</td>
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</tr>
<tr>
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<td>C/F</td>
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<td>0.9836</td>
<td>&lt;1</td>
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<tr>
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<td>F/A</td>
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<td>1.0486</td>
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</tr>
<tr>
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<td>C/C</td>
<td>0</td>
<td>1.0878</td>
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<td>0.5-2.0</td>
</tr>
<tr>
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<td>D/E</td>
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<td>1.2610</td>
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<td>0.5-2.0</td>
</tr>
<tr>
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<td>4.0704</td>
<td>1.0-6.0</td>
<td>4.0-6.0</td>
</tr>
<tr>
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<td>B/G</td>
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<td>5.0982</td>
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<td>4.0-6.0</td>
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<tr>
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<td>A/G</td>
<td>4</td>
<td>5.1160</td>
<td>1.0-6.0</td>
<td>4.0-6.0</td>
</tr>
<tr>
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<td>E/G</td>
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<tr>
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<td>8.0973</td>
<td>&gt;8.0</td>
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<tr>
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<td>C/D</td>
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<td>D/F</td>
<td>7</td>
<td>8.2430</td>
<td>&gt;8.0</td>
<td>8.0-12.0</td>
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<td>C/E</td>
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<td>B/D</td>
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<td>&gt;8.0</td>
<td>8.0-12.0</td>
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<td>6/16</td>
<td>B/E</td>
<td>2</td>
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<td>&gt;8.0</td>
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</tr>
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<td>5/16</td>
<td>A/E</td>
<td>1</td>
<td>9.3480</td>
<td>&gt;8.0</td>
<td>8.0-12.0</td>
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<tr>
<td>3/16</td>
<td>E/F</td>
<td>7</td>
<td>9.4668</td>
<td>&gt;8.0</td>
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<tr>
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<td>F/C</td>
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<td>11.4470</td>
<td>&gt;8.0</td>
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<tr>
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<td>A/A</td>
<td>0</td>
<td>16.4973</td>
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<td>&gt;16.0</td>
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<tr>
<td>4/18</td>
<td>C/A</td>
<td>5</td>
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<td>&gt;8.0</td>
<td>&gt;16.0</td>
</tr>
<tr>
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<td>B/A</td>
<td>2</td>
<td>30.2169</td>
<td>&gt;8.0</td>
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<tr>
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<td>A/F</td>
<td>6</td>
<td>30.8488</td>
<td>&gt;8.0</td>
<td>&gt;16.0</td>
</tr>
</tbody>
</table>
**Figure 20:** Haplotype network for *L. occidentale* workers collected from different sites throughout Riverside and San Bernardino Co. Black letters are the haplotypes, red numbers are the site numbers, and green numbers are the base pairs that differed.
Figure 21: Proportions of behaviors of *L. occidentale* workers toward each other for three distance categories between colonies

a) All behaviors
b) Behaviors grouped into not aggressive or aggressive
Figure 22: Proportions of behaviors of *L. occidentale* workers toward each other for five distance categories between colonies

a) All behaviors
b) Behaviors grouped into not aggressive or aggressive