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Title of Dissertation: "Morphometric and Molecular Analyses of the Sand Fly Species *Lutzomyia shannoni* (Dyar 1929) (Diptera:Psychodidae:Phlebotominae) Collected from Seven Different Geographical Areas in the Southeastern United States "

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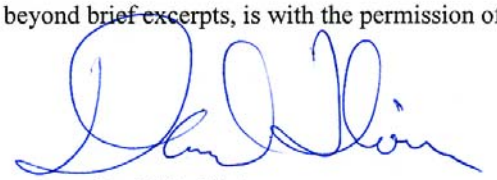

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ABSTRACT

Morphometric and molecular analyses of the sand fly species *Lutzomyia shannoni* (Dyar 1929) (Diptera: Psychodidae: Phlebotiminae) collected from seven different geographical areas in the southeastern United States

David A. Florin, Doctor of Philosophy, 2006

Dissertation directed by: Phillip Lawyer, Ph.D.
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Morphometric and molecular analyses were used to elucidate the variation among the sand fly *Lutzomyia shannoni* collected from seven widely separated locations in the southeastern United States: Baton Rouge, LA; Fort Bragg, NC; Fort Campbell, KY; Fort Rucker, AL; Ossabaw Island, GA; Patuxent NWRR, MD; and Suwannee NWR, FL. *Lu. shannoni* is a wide-ranging phlebotomine sand fly has been implicated in the transmission of a number of parasitic and viral pathogens of medical/veterinary importance. In light of this, it is imperative to answer the question of whether or not significant variation exists among the purported biogeographical populations so as to make a determination on the possibility of a cryptic species complex. A balanced approach to answering this question was emphasized by using the two-prong method of morphological and molecular data. The morphometric analysis entailed using univariate and multivariate techniques on a sample size of 40 males and 40 females from each collection site (with the exceptions, due to inadequate number of collected specimens, of Baton Rouge where morphometrics were not conducted for the specimens of either

gender and of Suwannee NWR where morphometrics were not conducted on the male specimens) A total of 54 characters from the male specimens and 49 characters from the female specimens were measured by an inserted micrometer in the ocular eyepiece of a compound microscope. Results indicate that while there is a certain amount of variation, it is not sufficient to discriminate among the collection sites. Two molecular markers, the mitochondrial DNA CO I and the nuclear DNA ITS2, were PCR-amplified and the resulting sequences compared. For both markers, the small amount of variation observed in the sequences did not have a diagnostic distribution and were not informative in distinguishing the specimens based upon collection site. There may exist one population of *Lu. shannoni* throughout the United States. In a corollary study, the population dynamics of *Lu. shannoni* were examined at the Patuxent NWRR, MD from June 23, 2005 to June 15, 2006 by conducting weekly light trap collections. The abundance pattern appears to be unimodal although only multi-year data can provide a definitive determination.

Morphometric and molecular analyses of the sand fly species *Lutzomyia shannoni* (Dyar 1929) (Diptera: Psychodidae: Phlebotiminae) collected from seven different geographical areas in the southeastern United States

by

David A. Florin

Dissertation submitted to the Faculty of the Department of Preventive Medicine and Biometrics of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 2006

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Chapter 1

General introduction

INTRODUCTION

Lutzomyia shannoni distribution, basic bionomics, and life cycle:

Lutzomyia (Psathryomyia) shannoni (Dyar) is a wide-ranging species of phlebotomine sand fly that has a known distribution from the state of Delaware in the United States south through Central America to northern Argentina (Young and Perkins, 1984; Young and Duncan, 1994). While a distribution spanning two hemispheres may seem impressive at first, there are sizeable gaps and discontinuities within this range. For instance, Young and Perkins (1984) noted the absence of the species in the state of Texas and northern Mexico. Lack of collecting data is partly suspected, but the authors also stated that the most likely reason is a void of the species preferred macrohabitat of extensive hardwood forests in the region. In the United States, anecdotal evidence from entomologists familiar with the collection of the species indicates that the range comprising a swath from the middle-eastern seaboard to the Mississippi river is largely fragmented and decidedly discontinuous with habitation zones generally associated with relatively undisturbed old growth, hardwood stands. Figure 1 details the known range of *Lu. shannoni* within the United States.

The basic bionomics of phlebotominae are concisely discussed in Lawyer and Perkins (2004) and Lane (1993). Readers unfamiliar with the subfamily should be aware that sand flies are small (about a quarter the size of a mosquito), the life cycle is holometabolous with 4 instars of larval growth occurring in ground litter or soil (and as such the egg, larval instars, and pupae are nearly impossible to collect in the field), strictly terrestrial, are found mainly in the tropics and subtropics, only females imbibe

blood, gender can easily be discerned from the external genitalia, and that most species exhibit a nocturnal to crepuscular activity pattern. Brinson et al. (1992) found *Lu. shannoni* in the northern hemisphere abundant for the 4-month period of May through August, with adults appearing in April and diminishing in November. The fourth instar of the species is presumed to be the overwintering stage (Comer, 1994) much as in *Lu. diabolica* (Lawyer and Young, 1991). In laboratory conditions, the species has the following life cycle: egg averaging 8.5 days, first stage larva averaging 9.6 days, second stage averaging 9.2 days, third stage averaging 11.8 days, fourth stage averaging 19.9 days (unless diapausing for the winter), pupa averaging 15.2 days, and adults averaging 8.6 days (Ferro et al., 1998).

Speciation and genetic studies of *Lu. shannoni*:

One of the major modes by which species originate is via "true speciation" that entails the processes of sympatric, allopatric and parapatric speciation (Solbrig and Solbrig, 1979). Sympatric speciation, generally considered the rarest, is divergence due to reproductive isolation preceding differentiation within the original range. Allopatric or geographical speciation is the process whereby the gene pool of a species becomes isolated, usually by forces of the physical environment that split the range into two or more parts hence separated populations which then undergo independent evolution or differentiation. Parapatric speciation, currently out of vogue with most evolutionists, is characteristic of organisms with limited dispersion capability and occurs when genetically different organisms are able to occupy different niches and maintain reproductive isolation.

One result of these speciation modes is the possible production of cryptic or sibling species. Mayr (2001) defined sibling species as "natural populations that are reproductively isolated from each other even though they often coexist sympatrically without interbreeding. Yet they are totally or virtually indistinguishable by traditional taxonomic characters." The term "traditional taxonomic characters" in Mayr's definition must be in reference to morphology, yet numerous publications have documented morphological variations between sibling species including sand flies (Marcondes and Borges, 2000; Marcondes and Alexander, 2003; Soares and Turco, 2003). Anez et al. (1997) used multivariate analyses to discriminate female morphologies of three species of sand flies in the *Verrucarum* group that were formerly considered to be essentially isomorphic and indistinguishable for the female gender. Even if two populations are morphologically identical, genetic differences are possible (and to a certain extent vice versa) as the relationship between phenotype and genotype are not linear (Solbrig and Solbrig, 1979)

Sand flies are considered to be generally poor fliers and therefore vulnerable to having populations undergo any of the modes of speciation. Alexander (1987) conducted a mark/recapture study using fluorescent powders on *Lu. shannoni* among other *Lutzomyia* species at a Colombian coffee plantation. Recaptured *Lu. shannoni* flies remained within 30 meters of the collection point although three males were recaptured at a distance greater than 100 meters within a time period of 24 hours after initial capture. Alexander (1987) summarized the data by concluding that sand flies do not generally range farther than 200 meters in a single night which is in general concordance with other studies on dispersal. Alexander and Young (1992) suggested that *Lu. shannoni* is a

relatively sedentary species due to possible lekking behavior in males and oviposition in close proximity to the tree bases by the females. When this limited flight range is compared to that of the muscoid flies that disperse more than 5 kilometers from breeding sites and more than 17 kilometers in a downwind direction (Mullen, 2002), sand fly populations appear to be especially prone to geographic isolation and subsequent genetic isolation. Depaquit et al. (2002) cited sand fly larvae developing in soil (poor medium for dispersal via zoohoria or phoresy) and adults flying very short distances, thus enabling the phlebotomine sand flies to serve as good models for examining variation in biogeography. The adult *Lu. shannoni* is a forest floor dwelling insect with vertical activity greatest from ground level to 0.5 meter (Brinson et al., 1992) and may simply not be prone to accidental dispersion by wind to the extent of preserving stable gene flow among isolated populations.

Given that this species is prone to speciation by possessing the attributes of low vagility and having a broad but very disjunct distribution, what may one see when examining field samples collected over a wide range? The first possibility, and essentially the null hypothesis of this study, is that there are no divided populations but instead one panmictic population extending all through the area of study – the flies are homogenous in both morphology and genetic make-up because they are all part of the same population. A second possibility is that the species may have undergone divergence but the manifestations in the genetic and/or morphological make-up is very subtle or non existent. Instead, other character attributes such as physiological or ecological are fundamentally different. Perhaps hybrids exist in some localities that are partially fertile intermediaries between two groups that have diverged to near reproductive isolation.

Another possibility is the existence of sibling species in the strict definition of Mayr (2001), where genetic divergence has occurred yet morphological constancy has remained. Finally, one might see significant morphological and/or molecular disparities between geographic populations. The salient point is that any species at any point of time is conforming to the pressures of natural selection and responding through a whole array of possibilities. The researcher may not be able to precisely quantify the state of the species, but instead can gather enough data on the taxon to hazard an approximate guess.

There are two publications on the genetic variability of *Lu. shannoni*. The first is by Cardenas et al. (2001) where polyacrylamide gel electrophoresis was applied to 13 isozyme loci from *Lu. shannoni* specimens collected from three widely separated locations in Colombia. The results were mixed. Genetic distances somewhat suggested isolated populations, yet the divergence of the distances was still well within the expectations for a single species. The inbreeding coefficients pointed to gene flow between the populations albeit at a restricted level. It is essentially left to the reader of the article to make the final conclusion although the authors hinted at possible inbreeding by subpopulations and low migration to explain the results. In the second published examination, Mukhopadhyay et al. (2001) compared allozyme frequencies of 20 enzyme loci from wild-caught flies within Colombia, a laboratory colony originating from Colombia, and a laboratory colony originating from Georgia, United States. [An interesting note is that the wild-caught flies which served to establish the Georgia colony may have been collected at Ossabaw Island (personal communication with L. Munstermann), a collection site of this study.] The authors cited three reasons for the interest in the population structure of the species: 1) broad, but disjunct distribution

favors divergence due to geographic isolation, 2) fragmentation of tropical rain forests have presented an opportunity to study reduced genetic variability due to genetic drift in an insect with diminished capability of dispersion, and 3) adequate collection of wild-caught specimens. The results indicated that three genetically distinct, geographically discrete groups could be discerned: two separate groups from within Colombia and the U.S. colony. Within Colombia, restricted migration between collection areas was indicated with some populations experiencing a virtual absence of migration or mixing of the genetic pool with other populations. If this degree of diversity can be found for populations inhabiting Colombia, one has basis to hypothesize that a similar sized and diverse land mass such as the southeast U.S. would also support the same diversity. Another interesting parallel is what effect the fragmentation of the hardwood/pine forests may have had on the genetic diversity of U.S. populations of *Lu. shannoni*.

A cryptic species complex of *Lu. shannoni* in South America may exist as the degree of morphological differences among other species in the subgenus *Psaythromyia* is quite subtle. Young and Duncan (1994) followed previous reports of placing *Lu. pifoni* as a junior synonym to *Lu. shannoni*. *Lu. microcephalus* is listed as a conspecific to *Lu. shannoni*. Two other species, *Lu. abonnenci* and *Lu. pestanai*, cannot be differentiated morphologically using the female keys. The genus *Lutzomyia* is suspected of having many cryptic species and the difficulty of producing a definitive key is summed up by the authors' comment: "Creating an unambiguous key to the subgenera and species groups of *Lutzomyia* is beset with problems." Further taxonomic research on these very similar sand flies may result in revisions to the current taxonomic classifications.

***Lu. longipalpis* studies and taxonomy issues:**

The current ambiguity in the taxonomic status of *Lu. longipalpis* has particular relevance to the question of sibling speciation of *Lu. shannoni*. Like *Lu. shannoni*, *Lu. longipalpis* has an extensive, but patchy distribution. Young and Duncan (1994) give the range as extending south from Mexico to Argentina, but as pointed out by Uribe (1999) there is a "considerable degree of geographical isolation exists between the various populations of *Lu. longipalpis,* which may be attributed to the interrelationships between its apparent limited flight range and geographical and climatic barriers (Lanzaro et al., 1993; Alexander et al., 1998; Munstermann et al., 1998)." When one examines different kinds of characters in turn and in combination, there appears to be a great deal of support for the consensus that *Lu. longipalpis* is indeed a cryptic-species complex. [Unlike, *Lu. shannoni*, there is a plethora of literature on *Lu. longipalpis*: morphological (De la Riva et al., 2001; Arrivillaga et al., 2000), physiological (Maingon et al., 2003; Yin et al., 2000), molecular (Lanzaro et al.,1993; Arrivillaga et al., 2003; Arrivillaga et al., 2002; Lampo et al., 1999; Uribe Soto et al., 2001), behavioral (Oliveira et al., 2001), ecologic (Ono et al., 2001), and geographic (Arrivillaga et al., 2002; Mutebi et al., 1998; Hodgkinson et al., 2003)]. Soares and Turco (2003) added to the above with a review citing studies showing differences in ultra structural morphology, salivary components, and parasitic infections between purported members of the *Lu. longipalpis* complex.

Uribe (1999) conducted a literature review of *Lu. longipalpis* and closed with a section devoted to molecular data and the forecast that this technique will address the issue of speciation in the taxon. Yet, this prediction may be risky. Molecular data can be viewed as a taxonomic character since it is an attribute that can be used in differentiating

taxa to some degree (Mayr and Ashlock, 1991). Greater predictive value is of course obtained when several kinds of taxonomic characters differ in the comparison. By what taxonomic characters do populations (or possible species) of *Lu. longipalpis* differ? Uribe listed several categories of variability: morphological, male pheromones and sexual behavior, isozymic variability, and molecular data. However, the scorecard for each character review does not point in the same direction. For instance, Uribe cannot reach a conclusion regarding the morphological data as a cited study comparing pale patches on the second and fourth abdominal tergites from certain adult male populations to others possessing only fourth abdominal tergite patches did not show concordance with follow-up laboratory crossings: the purported species produced viable offspring. The cited studies on male pheromones and sexual behavior show that in certain populations of *Lu. longipalpis*, males not only waft different pheromones but also display different behavior, a concrete step in reproductive isolation. The isozymic analyses are clouded by controversy over the genetic variability within and between natural populations and insectary colonies.

While the preponderance of the literature leans towards a species complex for *Lu. longipalpis*, the matter has not been officially resolved. Azevedo et al. (2000) conducted a morphometric and isoenzymatic study on four Brazilian populations with results indicating no significant morphological differences and genetic distances within the range of intrapopulational parameters. The authors pointed out that the traditional tool of morphology in taxonomic studies of *Lu. longipalpis* had been generally overlooked and that as far as the Brazilian populations are concerned, there is a “lack of detailed knowledge of morphological characters which might define different populations or

indicate some degree of intrapopulational heterogeneity." The taxonomic status of any organism should be derived from as many different kinds of characters as possible. Mayr and Ashlock (1991) provide six different types of taxonomic characters when differentiating taxa: 1) morphological 2) physiological characters 3) molecular 4) behavioral 5) ecologic 6) geographic. A morphometric analysis can serve to initially indicate the possible existence of biogeographical populations [unless, as pointed out by Mayr (2001), the morphologies of the sibling species are identical] with subsequent complementary analyses of other character attributes contributing to the conclusive taxonomic status.

Mayr and Ashlock (1991) made it clear that any claim of separate species where variational overlap occurs must be supported by more than one character analysis: "Closely related species are sometimes so variable and their variation is so overlapping that no single character seems to have absolute diagnostic value. A combination of characters usually permits the correct assignment of all seemingly intermediate specimens." Mayr (2001), in a subsequent publication, provided the especially pertinent commentary: "When it was discovered that the molecules that make up genes undergo evolution and have a phylogeny just like morphological characters, it was hoped that a definite phylogeny of organisms could soon be constructed; molecular evidence would enable a decision whenever the morphological data were ambiguous. Alas, things did not turn out to be quite so simple, for this reasoning ignored the phenomenon of mosaic evolution. Each component of the genotype can evolve somewhat independently of the rest of the genotype. Endeavors to construct phylogenetic trees on the basis of the evolution of one particular molecule frequently produced results that were clearly in

conflict with a massive amount of morphological and other evidence. For technical reasons, the molecules that were first used for such analyses were ribosomal RNA and mitochondrial DNA. Unfortunately, these molecules often went their own evolutionary way." While Mayr is for the most part explaining molecular evidence used to decipher phylogeny of the major animal groupings, what he stated can be applied here: molecular techniques, while tremendously valuable, are not panaceas and need to have the concordance of other character analyses; it is not the end all but instead another "character" in the differentiation of the species.

Molecular-based and morphology-based data in systematics research:

There are a number of studies that attempt to address the dichotomy between molecular-based and morphology-based data in systematics research. Baker et al. (1998) produced a phylogenetic version of a "Meta analysis", a methodical review of literature used in epidemiological studies to integrate the overall findings (Gordis, 2000). Fifteen systematics studies were examined that had used experimental models ranging from jellyfish to primates. For each taxonomic group, a molecular study and a morphological study were compared and then the data set interaction assessed. The authors found significant incongruence between the phylogenetic trees derived by the molecular data as compared to the trees derived from the morphological data. Yet even with this incongruence in methodologies, in nearly half the studies there was indication that both approaches yielded positive contribution to the combined phylogenetic determination. Morphological data was actually found to have more utility than molecular by virtue of higher consistency indices. The authors end the paper by refuting the claims that morphological studies are more prone to homoplasious effects (i.e. "independent

evolution" such as convergence, parallelism, or reversal) or that there is little utility for morphological work since the advent of molecular technology. In another study comparing mitochondrial DNA, nuclear DNA, and morphological data of the desert ants *Cataglypis* spp., Knaden et al. (2005) weaved all three approaches into a harmonious conclusion that centered on combining strengths from each analysis to reach an overall taxonomic decision. Malhotra and Thorpe (2004) combined molecular and multivariate morphometics in the identification of cryptic species of green pitviper snakes, *Trimeresurus* spp., and found that the combined approach revealed finer partition of the complex than was available by individual methods. The two-pronged molecular and morphological approach maximizes the information available to answer questions pertaining to systematics, each "prong" serving as a "proof" of the other method when concordance is reached.

Paquin and Hedin (2004) highlighted the necessity of using multiple types of biological information to create a balanced taxonomic approach when determining phylogeny of closely related species. The authors studied eyeless cave spiders belonging to the genus *Cicurina* that inhabit caves near central Texas. Unfortunately, commercial and residential development have exacted a heavy toll on the cave habitats resulting in placement of four geographically limited species on the Federal Endangered Species list. As such, these spiders have come under a great deal of scrutiny to delineate the precise range and to accurately estimate the size of remaining populations. The spiders do not lend themselves to easy analysis as very few adults are ever collected so identification must be made on the immature stages which display nearly identical morphology to other species of *Cicurina*. Molecular phylogenetic analyses would appear to be the easy and

sensible solution to this dilemma yet the authors discovered that sequences of the genetic marker of cytochrome oxidase subunit 1 mitochondrial DNA were inconsistent with predictions based on morphology. While acknowledging that their analyses may have revealed molecular introgression or the possibility of synonymous taxa, the authors emphasized that one-dimensional molecular taxonomy is fundamentally flawed by the absence of an external reference such as morphologic, behavioral, physiologic, ecologic, and geographic attributes. The authors argued that taxonomy based entirely on gene sequences can never observe incongruence "because the species tree *is* the gene tree"; cases where taxonomically valid species appear homogenous on a gene tree ignores other potentially differentiating characters. Dujardin et al. (1999) derived phenetic trees of Phlebotominae based on isozyme electrophoresis that were not in agreement with accepted classification and concluded that a true evolutionary approach springing from both morphometry and molecular studies was needed to conclusively solve the taxonomy question.

Vector incrimination of *Lu. shannoni*:

There have now been over 1,100 documented cases of cutaneous leishmaniasis caused by *Leishmania major* and 4 infections of visceral leishmaniasis with *L. infantum* among military service members who performed tours of duty in Iraq or Afghanistan. The actual number of cutaneous leishmaniasis cases is estimated to be higher with conservative estimates ranging between 1,500 – 2000 cases (Coleman et al., in press). Concern has been raised that returning infected service members may serve as a source of infection for domestic sand flies with an infection cycle becoming established in other hosts such as rodents and/or dogs. A hypothetical worst case scenario would unfold as

follows: A military installation within the U. S. receives troops returning from deployment with numerous individuals infected with *Leishmania* spp. Sand fly species native to the area become infected with the parasite while taking a blood meal from infected service members during an outdoor training exercises, family camp-outs, hiking, fishing, etc. The sand flies then vector the parasite to the rodent population during subsequent blood feeds. The infection becomes locally established and maintained in the natural rodent population with sustained enzootic cycles that periodically involve both dogs and humans by way of the infected sand fly vector. Eventually, additional foci of infection occur caused by the dispersion of infected rodents, dogs, or humans to other areas inhabited by sand flies. If other potential mammal reservoirs such as opossums and raccoons are factored into this simplified model, any derived outcome will be a major public health issue.

Research is currently underway to thoroughly study and assess the potential vector competency of sand flies in the United States for Old World *Leishmania.* If *Lu. shannoni* has also undergone a species complex divergence as purportedly in *Lu. longipalpis*, any vector competency study on *Lu. shannoni* will have to take into consideration the possibility of different vector competencies and/or disease manifestations in relation to geography or the cryptic species involved. Unequivocal identification of the purported vector is an absolute necessity in any epidemiological investigation as a cryptic species may exhibit different behavioral, ecological, and/or physiological attributes that are critical to the successful maintenance and transmission of the subject pathogen. Lanzaro and Warburg (1995) speculated that variability of *Lu. longipalpis* in different geographical areas produced variation in clinical manifestations

of leishmaniasis from the vectored parasite *L. chagasi*. The authors noticed that Central American infections were of the nonulcerative form of cutaneous leishmaniasis and the bites from *Lu. longipalpis* rarely produced long-lasting erythemas. Conversely, people bitten in Brazil and Colombia had the characteristic erythemas and also contracted the visceral form of *L. chagasi* even though the parasite isolates proved identical and *Lu. longipalpis* was the sole incriminated vector. Lanzaro et al. (1999) reported significant variation in the salivary peptide maxadilan, a salivary peptide which has vasodilatory and immunomodulatory effects on the vertebrate host, among biogeographical populations of the *Lu. longipalpis* complex. One of the most important malaria vectors in Asia, *Anopheles culicifacies sensu lato*, is recognized as a complex of five sibling species of which one is completely refractory to infection with *Plasmodium vivax* and partially refractory to *P. falciparum* (Adak et al., 2006). Adjami et al. (2004) summarized data showing sibling species of the black fly, *Simulium damnosum sensu lato*, having different vector capacities for different strains of *Onchocerca volvulus* with varying degrees of pathogenicity.

Miles et al. (1983) speculated that *Lu. shannoni* is most likely a vector of leishmaniasis in the central Amazon basin of Brazil due to catholic feeding patterns, being anthropophilic at times, and also feeding on known reservoirs such as the three-toed sloth (*Bradypas tridactylus)*. Christensen and de Vasquez (1982) found that sloths are actually the preferred mammalian host of *Lu. shannoni* and therefore the sand fly must be implicated in at least the transmission of parasites between these reservoir hosts. Other research has also lent support to the possibility that *Lu. shannoni* is a vector of *Leishmania* to humans but that due to very low rates of infection, the suspicion has not

been conclusively proven (Hashiguchi et al., 1992; Queiroz et al., 1994). *Lu. shannoni* is generally thought of as a forest dweller in old growth, relatively undisturbed forest. However, Jimenez (2000) reported that *Lu. shannoni* is among the species of sand flies that may be adapting to peridomestic environments (and possibly human hosts) due to deforestation of the natural habitat. In six rural localities in Acosta county, San Jose province, Costa Rica CDC miniature light traps were set in and just outside 72 houses during the study period. A total of 22 sand fly species were recorded with *Lu. shannoni* falling into the top eight of the most commonly collected. Azevedo and Rangel (1991) also collected *Lu. shannoni* in peridomiciliary areas of Brazil and found that the sand fly was attracted to both man and equines. A study in northern Venezuela confirmed that *Lu. shannoni* approach houses and may accidentally bite humans (Feliciangeli, 1987). Future studies assessing the potential of *Lu. shannoni* as a vector of *Leishmania* should take into account the environmental change and consequential behavior/host-seeking adaptations of the potential vector species.

Travi et al. (2002) evaluated the capacity of *Lu. shannoni* for vector competency of *Leishmania infantum*, the causal agent of canine visceral leishmaniasis and also human visceral leishmaniasis. Two dogs, differing by clinical status (oligosymptomatic and polysymptomatic), were used to infect the sand flies. (Unfortunately, the authors did not define exactly what criteria were used to classify the clinical status; presumably the polysymptomatic dog had active lesions while the oligosymptomatic did not.) *Lu. shannoni* was found to have a lower capacity to acquire the infection when compared to *Lu. youngi,* but the individual sand flies that did become infected actually harbored more promastigote parasites leading the authors to suggest that *Lu. shannoni* has a more

permissive condition for parasite development and could serve as a vector for visceral leishmaniasis in the presence of infected dogs.

Lawyer (1984) and Lawyer and Young (1987) showed that *Lu. shannoni* is a potential vector of *Leishmania mexicana* under laboratory conditions when 94.8% of a sand fly sample became infected after being fed on an infected Syrian hamster. Infection was noted to extend forward into the pharynx and mouthparts of the sand flies but more critically in the esophagus and stomodeal valve. These nonpumping organs, when massively infected with parasites, may facilitate transmission by forcing the sand fly to bite numerous times and/or creating a back pressure spewing parasites into a host when bitten. While the evidence pointed to the incrimination of *Lu. shannoni* as a potential vector of *Leishmania mexicana* to humans, the authors were careful not to state such because the five criteria of vector incrimination as outlined by Killick-Kendrick and Ward (1981) were not fulfilled. Paraphrased from the referenced document of Lawyer and Young (1987), the five criteria are: 1) anthropophilic vector, 2) distribution and sufficient abundance of the suspected vector match with the distribution of the disease, 3) the parasite can complete all of the required life cycle stages within the vector in either naturally or experimentally infected flies, 4) experimental demonstration that the parasite is transmitted from vector to host, 5) parasite isolated from wild-caught vectors and shown to be indistinguishable from the parasite causing disease in humans at that location.

Lu. shannoni now appears to fulfill four of the five criteria of vector incrimination since the Lawyer and Young (1987) publication. *Lu. shannoni* vector attributes for *Leishmania* include: anthropophilic/peridomestic behavior (Miles et al., 1983; Jimenez,

2000), distribution matching with a known disease location (Feliciangeli, 1987; Rangel et al, 1990; Azevedo and Rangel, 1991; Alexander et al., 1992; Rojas et al., 2004), ability of experimentally infected flies to maintain infection (Lawyer and Young, 1987), and the ability to transmit the infection experimentally (Lawyer and Young, 1987). The final verdict is not in because the fifth criterion of parasite isolation from wild-caught *Lu. shannoni* at a human disease location is very elusive as infection rates in the sand flies may be extremely low (Hashiguchi et al, 1992). Rowton et al. (1991) appear to have come exceedingly close with the report from Guatemala of unidentified flagellates found within *Lu. shannoni* having matching isoenzyme patterns to unidentified flagellates from a patient with mucosal lesions. Confirmation of *Lu. shannoni*'s ability to transmit *Leishmania* in nature may be further delayed due to the overlap with better studied and more numerous vectors in endemic areas such as *Lu. longipalpis* that ultimately shifts the focus of research away from this species.

Ferro et al. (1998) cited unpublished data on experimental infection of *Lu. shannoni* with *L. panamensis. Lu. shannoni* has been shown to harbor other species of flagellates (Arias et al., 1985; Rogers et al. 1988), but these may be of no major human or veterinary importance. Regarding viral agents, *Lu. shannoni* is a vector of the swine disease New Jersey serotype vesicular stomatitis (Comer et al., 1992). It is likely, given the extensive range and broad host preferences of the species, that more pathogens/parasites will be discovered using this sand fly as a transmission vehicle.

Research goals:

This research was designed to examine the sand fly species *Lu. shannoni* collected from seven disparate locations in the United States and determine if there is enough

morphologic and molecular variability to indicate the existence of biogeographical populations. Simply stated, there are two hypotheses being tested: 1) the null hypothesis that there is no significant difference between the biogeographical populations and in effect the sand flies collected from all the collection sites are homogenous units of one population (albeit within normal individual or group variation), or 2) the alternative hypothesis that there exists significant differences that could indicate cryptic species formation. It should be noted that the term "population" is used throughout this document to signify *Lu. shannoni* from individual collection sites. The biological concept of a population is the sharing of genetic material, a gene pool, among organisms of the same species (Pianka, 1983). There may in fact be a single population of *Lu. shannoni* throughout the entire U.S. or conversely a multitude of separate populations. That is one of the central issues that this research addresses. The reader needs to be aware that "population" as used to describe all the individuals of a sand fly species in a given area is not the same as the strict biologic definition.

A balanced approach to testing these hypotheses incorporates morphological and molecular data. However, even if this research is found capable of testing the above hypotheses, a final determination of the homogeneity or hetereogeneity of the biogeographical populations can only be answered by additional studies that explore physiological, behavioral, ecologic, and geographic attributes. Combining this study with subsequent research may provide enough information for a sound taxonomic determination. With that stated, the goals of this research are to examine the differences and variability between disparate populations by the following: 1) assess the morphological differences, 2) assess the mitochondrial DNA Cytochrome c Oxidase

subunit 1 variability, 3) assess the ribosomal DNA Internal Transcribed Spacer 2 variability, and [in a corollary to the previous three goals] 4) determine the population dynamics of *Lu. shannoni* inhabiting the Patuxent National Wildlife Research Refuge (NWRR), MD in relation to seasonal and weather conditions.

Protocols and dissertation format:

The laboratory studies were conducted in the Immunology Department, Uniformed School of University of Health Sciences, Bethesda, MD. All pertinent laboratory safety and environmental protocols were adhered to while conducting this research. Field collections on the military installations of Fort Bragg, NC; Fort Campbell, KY; and Fort Rucker, AL were approved by proper authorities within the Natural Resources/Environmental offices of those respective installations. Field collections at Baton Rouge, LA; Patuxent NWRR, MD; and Suwannee NWR, FL; and were approved under existing permits and with the consent of the following permit holders respectively: Louisiana State University; USUHS; and Dr. D. Kline, University of Florida. Permission for field collections at Ossabaw Island, GA was granted by the Ossabaw Island Foundation to Dr. E. Rowton, Walter Reed Army Institute for Research.

This dissertation is presented in chapters that are largely aligned with the research goals. Each research chapter (2 - 6) was written and organized in a manner that would facilitate its publication in a peer-reviewed journal. Format of this document conforms to the guidelines promulgated in the USUHS "Graduate Student Thesis and Dissertation Requirements Preparation Manual, 10^{th} edition."

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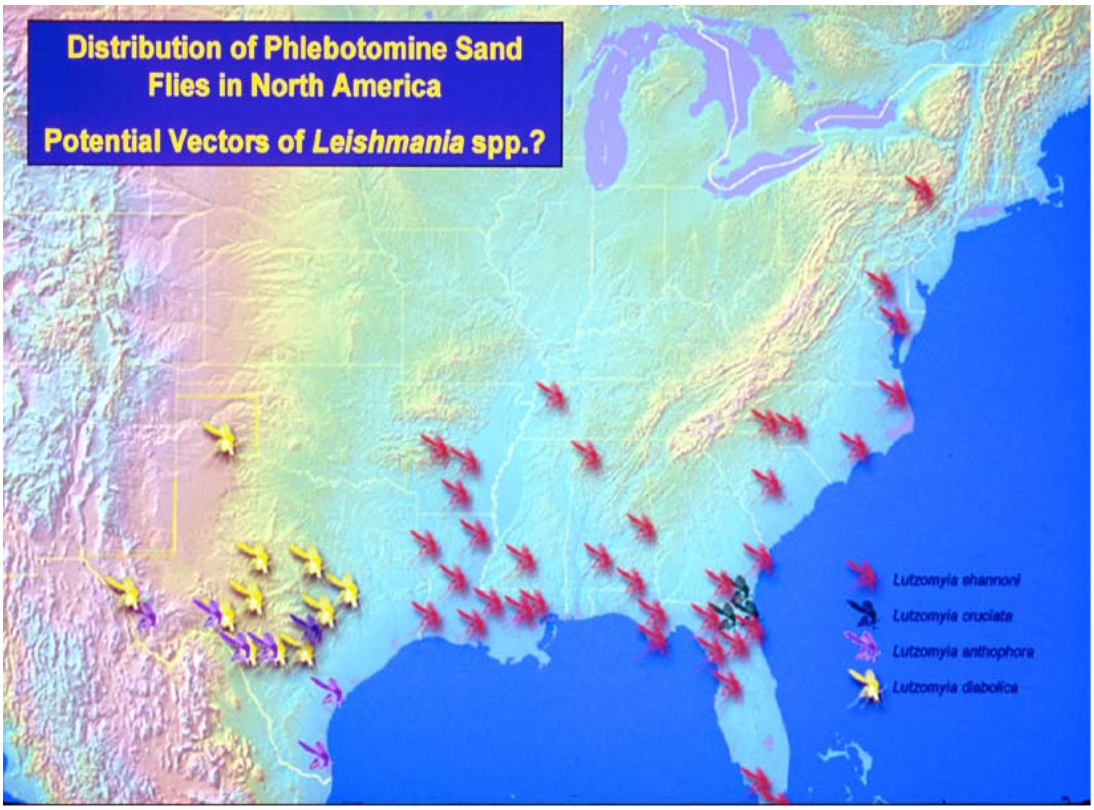

Figure 1. Distribution of *Lu. shannoni* and 3 other species of phlebotomine sand flies in North America.
Source: E. Rowton, WRAIR

Chapter 2

Morphometric analyses of the sand fly species *Lutzomyia shannoni* (Dyar 1929) (Diptera: Psychodidae: Phlebotiminae) collected from six different geographical areas in the southeastern United States

ABSTRACT

A morphometric study of adult male and female specimens of *Lutzomyia shannoni* (Dyar) collected at 6 different locations within the southeastern United States was conducted to assess if grouped specimens from each location could be differentiated on the basis of certain morphological features. The collection locations within the United States were: Fort Bragg, NC; Fort Campbell, KY; Fort Rucker, AL; Ossabaw Island, GA; Patuxent NWRR, MD; and Suwannee NWR, FL. Forty females and forty males from each location (with the exception of Suwannee NWR where the male morphometric analysis was not performed due to insufficient number of males collected) were analyzed morphometrically from 54 and 49 characters respectively. Univariate and multivariate analyses indicate that while there is variation between the groups from each collection site, it is not sufficient to completely separate the groups into distinct populations. The grouped specimens from each collecting site are within the normal variance of the species indicating a single population in the southeast U.S. with adequate gene flow in the past and/or present to maintain homogeneity. It is emphasized that the conclusion of homogeneity is based on the single character analysis of morphological features. Additional character analyses of *Lu. shannoni* based on molecular, behavioral, ecological, and physiological characteristics are necessary before ruling out the possibility of cryptic species.

INTRODUCTION

Size does matter, as seemingly inconsequential variation in size can cascade into major structural and performance diversity within a population directly affecting the fitness of the affected population and perhaps the entire species (Koehl, 1996). Natural selection may favor one shape or size in a certain population but could be relaxed in another population where environmental conditions are different. If a particular size variation in a morphological feature does not have a major bearing on the fitness of the organism, then a great amount of variation may be observed as the now "liberated" morphological variations radiate out from the population to be the incipient seeds of cryptic species formation. In either extreme and throughout the range of fitness pressure, size and resultant shape of morphological features are not only measurable quantities of divergence, but may be the impetus that spurs a faster rate of divergence among other taxonomic characters.

While size is certainly important, defining separation of a purported sibling species on the basis of size differences among a few characters could be risky. In the first place, one must take into account the normal size variation of the species and the examined population. What is significant to a statistical program could actually be meaningless in nature. Size should be examined in context with other character information such as reproductive structures, internal morphology, embryo characters, metabolic factors, immunological factors, molecular characters, courtship behavior, habitats, biogeographic distribution, etc. Dujardin et al. (1999) speculated on possible cryptic species within *Lu. runoides* based on the male genital filament lengths and in a more recent paper, Dujardin

et al. (2005) claimed the existence of two separate morphotypes of *Lu. aragaoi* primarily based on the shape of the paramere in males. In both of these studies, the authors were very careful not to post the announcement of a newly found separate species but instead questioned the homogeneity of the species defined by previous classification. The term "speciation process" is used often in peer-reviewed journal articles on morphology and gives one the impression that a major challenge facing morphology-based taxonomy is the temporal nature of speciation. Taking size measurements of a population is a "snap shot in time" as change can be quite rapid in certain species. If a researcher notes significant size difference between two populations, there is usually no information available to judge just when the differences became evident. Have the size differences existed for thousands of years? Hundreds of years? Or perhaps the size variations popped up over the last few generations? Perhaps the variation is seasonal? What is the time frame in which the size variation impedes reproduction among populations or facilitates niche separation? Adding to the temporal quandaries are other considerations such as size variation being a passive consequence of temperature, habitat, nutrition, parasite load, etc.

Evolution can mold an organism one way and then fluctuate over time to reverse the process as Weiner (1994) eloquently stated in the Pulitzer prize-winning book *The Beak of the Finch.* Documenting the research of others using "Darwin's finches" of the Galapagos Islands, Weiner described how oscillations in character size, such as beak width, occurred from selection pressure responding to environmental change. A phase favoring wider bills may last for a decade or more and then swing back to a decade-long phase favoring narrower bills. These oscillations in character size create "fusions and

fissions" of the overall morphotypes as hybrid forms dominate at certain times and virtually disappear at other times. The formation of hybrids, characteristic of parapatric speciation, has generally been refuted by contemporary evolutionists as a very marginal mode of speciation in animals (Mayr, 2001). However, Weiner addresses this by saying: "Traditionally, evolutionists have thought of this kind of intermixing and rapid evolution as the more or less exclusive property of the plant kingdom. Mayr concluded that hybridization was unlikely to play much of an evolutionary role among higher animals. Yet that may not be true. Certainly it is rarer among animals than plants, but among birds and many other groups of animals, it seems, hybridization is widespread. It is common in toads of the large genus *Bufo* and in many families of insects. It is extensive among fish, which usually spread their sperm and eggs in the water to be fertilized outside their bodies, rather like plants."

Rangel et al. (1996) suggested that *Lu. whitmani*, a South American sand fly, was composed of at least two geographically cryptic species based on three characters: consistent difference in the ratio of the length of genital filaments to the genital pump, differential peridomestic attributes, and different species of *Leishmania* vectored by each geographic population. Dias et al. (1999) collected *Lu. whitmani* at five different locations in Brazil and analyzed the specimens morphometrically for 42 characters in females and 37 characters in males. The sample numbers were small, as only 15 specimens of each gender at each location were examined. While the statistical data were insufficient to discriminate among all five populations, certain populations could be distinguished from the other four populations based on pair-wise multiple comparison procedure if shown significant in one-way analysis of variance. Further phenetic analysis

coded the character states as binary properties depending on the relationship of the subject character's mean value in a population compared to the mean value of the entire five populations. The phenograms produced for each gender indicated that two biogeographical groups of the species existed in Brazil, although the authors stated that the taxonomic status could not be defined by the study alone. Margonari et al. (2004) followed up on the above morphometric study with RAPD-PCR analysis of four of the five populations. The resulting phenograms produced were in partial concordance with those derived from the previous morphometric survey and provided additional evidence of divergence within the species.

In a frequently referenced work of contemporary morphometrics, Klingenberg (1996) defined allometry as the variation between organisms based on size variation. Three levels of allometry are introduced: static, ontogenetic, and evolutionary. Static allometry is simply size difference due to variation among individuals in the same population and age group. Ontogenetic allometry, as the name implies, deals with variation exhibited by the growth process. Evolutionary allometry is based upon variation of morphological characters resulting from evolutionary processes across the studied phylogenetic branch. It is evolutionary allometry that is targeted in a morphometric analysis designed to test the null hypothesis of a homogenous population or the alternative hypothesis of a cryptic species complex. The researcher attempts to exclude the simple static and/or ontogenetic allometric differences of the studied taxa to compare only the size variation that has resulted due to phylogenetic divergence.

Dujardin and LePont (2004) asked the following question that is central to this study: "Is a single species metrically recognizable in spite of its geographical variation?"

Using ten sand fly species from Latin America including *Lu. shannoni*, the authors compared populations of each species within and across ecogeographic regions defined by ecological parameters. Particular effort was made to exclude possible interference due to simple size variation or allometry by removing allometric trends with the use of a common principle component model. The procedure is explained as follows: "In this model, a single component is taken to account for shared allometric variation. In the space of the log-transformed measurements, conspecific individuals are expected to be found along the straight line defined by this single component. Metric variation orthogonal to this direction is allometry-free by construction, describing metric differences independent from size variation." Within the ecogeographic regions the results indicated that size-free divergence was not significant. However, there was significant size-free divergence noted between ecogeographic regions especially when altitude was considered. Thus, the authors concluded that metric differences of morphological characters between ecogeographic regions are valid if a size-free analysis is conducted first which removes the variation (size) that may be merely a passive consequence of differences in temperature, nutrition, stress, competition, parasite burden, etc. in one particular region compared to another.

A literature search and review reveals one published study pertaining to the morphometric features of *Lu. shannoni* collected within the United States. The lone study is cited by Young and Perkins (1984) who stated the following: "Both sexes of *L. shannoni* from the USA and neotropics (from southern Mexico to northern Argentina) are remarkably similar in structure. There is little or no morphological variation (Rozeboom 1944)." Yet, a close examination of the Rozeboom (1944) publication raises serious

doubts as to the validity of the above citation. L. E. Rozeboom reported the collection of 9 sand flies, 4 males and 5 females, from a location near Florence, Alabama. These 9 specimens were compared to an unmentioned number of the purported same species collected in Brazil. For each gender, the following characters were measured: Palp I, Palp II, Palp III, Palp IV, Palp V, Wing alpha, Wing beta, Wing gamma, Wing delta, ratio of Wing alpha/ Wing beta, and spermathecae length (females only). The range of measurements from each location were then placed side-by-side for viewing yet no statistical compilations were conducted. The extremely small sample size, limited number of characters examined, and absence of meaningful statistical analysis places the belief that there is no morphological difference between the two geographic populations on very tenuous ground. There appear to be no documented studies examining morphometric variation of *Lu. shannoni* specimens collected from within the U.S. and one gains the impression that *Lu. shannoni* in other areas such as Central and South America has generally been passed over in entomology research for the more competent *Leishmania*-vector species such as *Lu. longipalpis* or *Lu. whitmani*.

MATERIALS AND METHODS

Collection site data:

Sand flies were collected in dry ice-baited John W. Hock Company New Standard Miniature Light Trap Models 1012 set just prior to dusk and allowed to run continuously until collection of the specimens during the following morning. The number of traps used per night at any one site varied from 4 to 20: four traps were used one night/week during the trapping period at Patuxent National Wildlife Research Refuge while the number of traps set nightly ranged from 15 to 20 for all other collection areas. A capture of at least 50 males and 50 females was considered sufficient to cease collection efforts at a particular location [with the exception of the Patuxent National Wildlife Research Refuge where a seasonal prevalence study was conducted (Chapter 6)].

Field collections were made at the following six locations in the southeast United States:

- Fort Bragg, North Carolina: A total of 143 sand flies were successfully processed of which 129 (54 males and 75 females) were identified as *Lu. shannoni* and 14 identified as *Lu. vexator.* (The term "successfully processed" is used here to denote a specimen that was dissected, slide-mounted, and in proper sample integrity or condition to be capable of deriving the identification to species.) All specimens were collected from September 1 – 3, 2005 at the 14,000-acre Fort Bragg Army installation located near Fayetteville, NC. The collection sites were mostly situated along fire-break roads that paralleled military training areas and as such were heavily modified by human use and disturbance. Selective loggings,

controlled burns, and replantings of the pine tree cover have been standard practice for well over 20 years on much of the installation's training grounds. This terrain is flat and sandy, with extensive stands of pine and sparse understory.

- Fort Campbell, Kentucky: A total of 155 sand flies were successfully processed of which 132 (79 males and 53 females) were identified as *Lu. shannoni* and 23 identified as *Lu. vexator.* All specimens were collected from September 6 – 8, 2005 at the 114,000-acre Fort Campbell, Kentucky Army installation that is split nearly equally between the states of Kentucky and Tennessee. Collection sites were characterized by hilly terrain and deciduous tree cover interspersed with grassy meadows.
- Fort Rucker, Alabama: A total of 127 sand flies were successfully processed of which 122 (55 males and 67 females) were identified as *Lu. shannoni* and 5 identified as *Lu. vexator.* All collections were made on the 58,000-acre Fort Rucker Army installation, which is dominated by longleaf pine-scrub oak-wire grass ecosystems. Collections were conducted during two separate periods: May 24 – 25 and September 10 – 12, 2005 with all but 21 specimens collected on the latter trip.
- Suwannee National Wildlife Refuge (NWR), Florida: A total of 57 sand flies were successfully processed of which 55 (2 males and 53 females) were identified as *Lu. shannoni* and 2 identified as *Lu. vexator.* Collections took place on the 53,000 acre federal wildlife sanctuary during two separate trips: May 27 – 29 and September 13 – 16, 2005. All the

specimens that were processed for the study were collected on the earlier trip. The collecting trip conducted in September produced another 83 sand flies, but unfortunately only 4 males were among the total. Therefore, the entire collection of the second trip was not processed as enough females had been procured during the earlier trip. Due to only 6 male specimens being collected in total during the two collecting trips, this site was excluded from the male morphometric analysis. Natural salt marshes, tidal flats, bottomland hardwood swamps, and pine forests are dominant features of the refuge; the latter two were targeted as trap locations.

- Ossabaw Island, Georgia: A total of 110 (50 males and 60 females) *Lu. shannoni* were processed; no other species of sand fly was collected. All collections took place on the 126,000 acre coastal barrier island located approximately twenty miles south of Savannah. The uplands are characterized by deciduous oak and second growth forest interspersed by creeks, rivers, and tidal marsh. A single collecting trip was conducted that took place September 19 – 21, 2005.
- Patuxent National Wildlife Research Refuge (NWRR), Maryland: A total of 99 (41 males and 58 females) sand flies were processed from this 12,750 acre wildlife refuge located off the Patuxent river between Baltimore, MD and Washington, District of Columbia. All collected sand flies were identified as *Lu. shannoni*. The habitat can be characterized as forested with patches of grassland broken up by numerous wetland impoundments. Consistent collections occurred at this site on a weekly

basis from June 7 – November 6, 2005 and from March 31 – June 15, 2006 (detailed in Chapter 6).

- Baton Rouge, Louisiana: A total of 14 sand flies from a single collection trip (May 20 – 22, 2005) were successfully processed from this site with 13 (2 males and 11 females) being identified as *Lu. shannoni* and 1 as *Lu. vexator*. This location actually included three collecting sites: two within Baton Rouge proper and one site located in Baker, LA approximately 10 miles north of Baton Rouge. The collection site in Baker was Greenwood State Park where numerous freshwater fishing ponds were bordered by deciduous thickets with saw palmetto/ brier understory. The Baton Rouge sites were an undeveloped lot in a commercial sector and a wetland thicket adjacent to a high school. All three sites were small in size compared to the other collection sites and all were heavily impacted by commercial development and nearby residential activities. Due to the low number of specimens collected from all three sites (region was in a drought at time of collecting trip), this location was not included in the morphometric analysis, however the thirteen specimens were utilized in molecular studies (Chapters 3 and 4).

Global Positioning System coordinates were documented for all light trap locations and are available upon request.

The major considerations in the selection of the above sites were to obtain distant representative points throughout the documented range of the species within the U.S. and to sample in different ecoregions of the range. The geographical distance spread and the

different ecological conditions of the various collecting sites increases the chances of detecting any populational discrepancies that may exist within this species (Solbrig and Solbrig, 1979). The various sites are situated in three different ecological provinces of the southeastern U.S. (USDA Forestry Service, 2006). Figure 1 displays the approximate location of the collection sites and details the ecoregions mapped out according to dominant vegetational/ecological communities inhabiting the delineated zones. The sites of Patuxent NWRR, Fort Bragg, Ossabaw Island, and Suwannee NWR are located in the Outer Coastal Plain Mixed Province with dominant trees being evergreen oaks and members of the laurel and magnolia families that thrive in the sandy soils and abundant rain fall of the province. The Fort Rucker site straddles the border delineation of the Outer Coastal Plain Mixed Province and the zone designated as the Southern Mixed Forest Province. The Southern Mixed Forest Province is characterized by stands of loblolly pine, shortleaf pine, and other southern yellow pine species. Fort Campbell is essentially the northwestern extreme of *Lu shannoni*'s range and falls within the Eastern Broadleaf Forest (Continental) Province dominated by broadleaf deciduous forest, with the unique drought-resistant oak-hickory association adapted to the smaller amounts of precipitation.

Preparation and character measurements of specimens:

After collection, all specimens were immediately preserved in 100% ethanol for transport back to the USUHS laboratory. In the laboratory, the sand flies were prepared for mounting on glass slides by placing each specimen in a small Petri dish flooded with 100% ethanol. Four structures were dissected from the body: head, terminal 6 segments of the abdomen, one hind leg, and one wing. The remainder of the specimen was placed

in an individual Nalgene® Cryoware™ cryogenic vial containing 100% ethanol, assigned a designated accession number that matched the number assigned to the dissected parts, and then stored in a -70 degrees Celsius (º C) freezer for future use in genetic sequence analysis. The dissected structures were cleared for a period of 48 hours at room temperature in specimen clearing fluid (BioQuip Products, Inc. #6373A). After clearing, the four structures were centrally mounted on pre-cleaned 3" X 1" (1.0 mm thick) glass slides in mounting medium (BioQuip Products, Inc. #6371 PVA). An 18-mm microscope glass cover slip was placed over the mountings and the slide then placed in a drying oven set at 45º C for five days. Upon removal from the drying oven, the cover glass was "ringed" with an epoxy to produce a permanent mount. From the mounted structures of the abdomen (genitalia) and head, the sand flies were identified to gender and species using the taxonomic keys of Young and Perkins (1984).

A total of 200 male and 240 female *Lu. shannoni* were used in the statistical analyses of this study. The goal was to have a uniform processed sample size of 40 specimens of each gender from each collection site. As described above, an inadequate number of males were collected at the Suwannee NWR site and therefore this collection site was excluded from the analyses of the male data. The criteria for choosing the specimens to be used were based on completeness and integrity of the dissected, slide-mounted features. As in any study using insects collected from light traps, a certain amount of damage to the delicate, external features was inevitable and unavoidable. The target collection goal of 50 males and 50 females per site was the minimum; for most of the sites well over 130 sand flies were actually processed and then 40 males and 40 females were selected on the basis of completeness and visibility of the characters to be

measured. This selection process may have introduced a selection bias into the study as certain specimens that were excluded may have had attributes such as small size that predisposed them to poor visibility on mounted slides or conversely, larger-sized specimens may have been more prone to damage by the intake fan of the light trap. However, this potential bias was viewed to be of a relatively minor nature as only those specimens that were severely damaged (e.g., incomplete head or fragmented abdomen) were excluded. No attempt was made to exclude specimens with secondary damage such as loss of flagellomerers, palp segments, leg, or wing.

The morphological characters used by Dias et al. (1999) in the morphometric study of *Lu. whitmani* and by Azevedo et al. (2000) in the study of *Lu. longipalpis* were the foundation for the characters chosen in this study. In addition, correlation of the characters used for identification to genera, subgenera, and species by Young and Perkins (1984) and Young and Duncan (1994) to the selected characters provided a confirmation of taxonomic importance. All morphological characters examined in both genders were external features with the exceptions of spermathecae length, width, and ratio measured in the females. The morphological characters were measured by using an micrometer inserted in one of the two 10X ocular eyepiece lens of a calibrated American Optical Corporation Series 150 compound microscope with measurements recorded from the 4X, 10X, and 45X objectives, depending upon the size of the character. The same researcher measured and recorded all data throughout the study. A series of raw measurements were compared to measurements obtained from a different researcher, with the correlation result of 99.95% obtained between the two sets of measurements.

Table 1 provides a list of the morphological characters used in this study and a description of how the measurements were derived. Sand flies, being sexually dimorphic insects, require separate morphometric analyses by gender even though the majority of the characters used in this study were common to each sex. The total number of characters initially assessed was 67 for the males and 62 for the females. It is important to mention again that these sand flies were collected in light traps and many of the specimens were damaged from being struck by the intake fan, jostled with other insects in the collecting net, adhering to the net because of moisture, etc. Delicate, distal features such as antennae, tarsi, palps are more prone to damage or breakage and that is why only the first ten flagellomeres (vice the full fourteen) of one of the antennae were included in the study. In the course of recording measurements from the compound microscope, if a character was found to be absent or so severely damaged that the researcher did not believe a viable measurement could be made, no data was recorded for that item. Any character that exceeded more than 10% of cases with no entries was excluded from the study. Therefore, the following characters from both genders were not used in any analyses: labrum width, labrum ratio, palp V length, palp V width, palp V ratio, flagellomere VIII length, flagellomere VIII width, flagellomere VIII ratio, flagellomere IX length, flagellomer IX width, flagellomere IX ratio, flagellomere X length, flagellomere X width, and flagellomere X ratio. Thus, the total number of characters entered into the analyses of the males was 54 and for the females the number was 49. Included in these totals are a number of length/width ratio measurements conducted on certain characters. The ratios are designed to reduce the confounding of simple size differences in the sample and as a comparison in the ordination analyses.

The micrometer measurement data were converted into real measurements of micron units using the calibration values of the ocular micrometer in relation to each objective lens as outlined in Ash and Orihel (1997). The software package SPSS® 12.0.1 for Windows was used for all statistical aspects of the univariate and multivariate analyses.

Univariate analysis:

Assumptions of an analysis of variance (ANOVA) model are that the observed differences constitute a simple random sample, populations are approximately normally distributed, and the variances of the populations are equal (Daniels, 1999). The ANOVA is a robust analysis that can usually withstand minor violations of the assumptions to the model (Daniel, 1999; Remington and Schork, 1970). The characters entered for analysis were screened for a normal distribution pattern by using two methods: quantile-quantile plots and probability plots. The plots from each method indicated that all characters, including the ratios, are of a normal distribution as evidenced by the data points clustering around a straight line (plots not shown). Boxplots of each character were examined for outliers with the majority showing none. The few outliers detected were checked for accuracy by re-examining specimens from which the measurements were derived from. If the measurements were accurate, the "outliers" remained in the data. Test of homogeneity of variances (not shown) for significantly different variances as determined by a Levene statistic p-value of significance less than 0.05, did indicate that some characters displayed unequal variances between the populations. Since the Levene statistic can be overly sensitive in some applications, the standardized residuals, the difference between the observed value minus the predicted value all divided by standard deviation (Kleinbaum et al., 1998), were examined graphically. The characters indicated

to have unequal variances by the Levene statistic were found to be within an acceptable range when the standardized residuals were plotted against the collection location as the spread of the variances did not exceed 3 standard deviation units above or below the mean. A robust ANOVA was therefore assumed as the unequal variance violations appeared minor and normal distribution had previously been established. The ANOVA compared the character means from each collection location (male and female separate) and then conducted a pairwise multiple Bonferroni comparison test to determine which means were significantly different at the alpha 0.05 level. Those character means that were found to be significantly different were then applied to a phenetic analysis.

Phenetic analysis:

A phenetic analysis, similar to that outlined in Dias et al. (1999), was conducted. The grouped specimens from each collection site were considered as 1 operational taxonomic unit. A mean value (M1) was derived for each character in a given operational taxonomic unit and then an overall mean value (M2) was calculated by averaging the means across the collecting sites for that particular character. Coding was based on the relationship between the taxonomic means (M1) and the overall means (M2) and proceeded as follows: a code of “0” was assigned to any relationship where M1<M2, and a “1” for any relationship where M1 = or > to M2. Binary matrices for each gender were constructed and then entered into a hierarchical cluster analysis using the simple matching method for binary data and the between-groups linkage cluster method. The similarity matrices among the gender separated populations were then incorporated into phenograms depicting the similarity in regards to the collecting sites.

Multivariate analysis:

Ordination methods, such as canonical discriminant analysis (CDA) and primary component analysis (PCA), are multivariate techniques by virtue of combining several different categories or variables of measurements to make an assessment of classification (Afifi and Clark, 1984). The appeal of ordination techniques is nicely summed up by Foottit and Sorenson (1992): "Due to the great diversity of arthropod species and their morphological variability, taxonomists are faced with a daunting task when they attempt to array insects into manageable systematic groups. Often the response of an organism to selective forces will manifest itself as the adaptation of a number of features to many interdependent biological and environmental factors. Morphologically, responses may occur in a multidimensional fashion, rather than as a change in a single character (Blackith, 1960; Blackith and Reyment, 1971; Gould and Johnston, 1972; Sokal, 1986). Populations and species may overlap when characters are studied individually, but they may become distinct entities when many characters are considered jointly." Unlike cluster analysis, which emphasizes overall similarity of the groups, in ordination the inherent covariance patterns are discerned. CDA in effect reduces or simplifies the differences between populations by determining the optimal subset of variables that differentiate or "discriminate" the population groups.

Canonical discriminant analyses (CDAs) were conducted on the separate male and female data. Missing values in the data from any individual fly were replaced with the respective serial mean since ordination techniques can be sensitive to inequality of data entries (Pimentel, 1992). Assessment of the model in each analysis was accomplished by examining the Wilks' lambda and the Wilks' lambda difference tests (Garson, 2006).

The Wilks' lambda tests the significance of the discriminant function as a whole with a significant lambda signifying that the rejection of the null hypothesis of the groups having the same mean discriminant function scores. A significant finding therefore enables one to conclude that the model is discriminating. The Wilks' lambda difference test, basically an F test of significance of the ratio of two Wilks' lambda, is another tool used in this study to assess model functionality of the canonical discrimination analysis. In measuring the strength of relationships, the canonical correlation, equivalent to the Pearson's correlation of the discriminant scores with the grouping variable, was examined. This value can range from -1 to +1, with a perfect positive linear relationship being +1 where all the variance in the discriminative scores is attributed to group differences (Garson, 2006). Assessment of the degree of separation of grouped specimens by collections sites was made through the classification of the original data and by leave-one-out-cross-validation. Classification of the original data essentially entails re-computing each individual sand fly against the total analysis and tallying the percent of correct groupings. Cross-validation produces unbiased estimates (Afifi and Clark, 1984) by deriving the functions from one sample and applying it to another. Leave-one-out-cross-validation basically removes a measured point (individual sand fly specimen) from the data set and then estimates to what grouping (collection site) it should be placed into based on discriminant scores. This procedure is repeated for every measured point and produces a stronger classification since the measured points are not part of the data during the analysis as in the original data classification method. A number of different, but separate, analyses were conducted in order to assess the optimal discriminant model of the data: character data in microns, log-transformed character data,

ANOVA significant characters, ANOVA significant log-transformed characters, ratios, log-transformed ratios, significant ratios, log-transformed significant ratios.

A “size-free analysis” was modeled after Dujardin and Le Pont (2004). A detailed summary of theory and calculations supportive of this method can be found in Klingenberg (1996). For each gender, a multigroup PCA was computed from the variance-covariance matrix. The width and ratio measurements were not entered into the analysis since the standardized dispersion matrices indicated that the homoscedasticity were generally further dispersed than in the other characters. Missing data values from any individual sand fly were replaced with serial means. All data entered into the PCA was log-transformed to make the variances more homogenous and relations among the variables more linear (Klingenberg, 1996). Those characters in an orthogonal projection from the single component of the data cluster line were viewed as allometry-free by construction and then submitted to a canonical discriminant analysis. Determination of orthogonal projection was assumed when a character displayed low correlation to the first principle component axis. Conversely, any character exhibiting a high correlation to the first principle component axis was assumed be inherently prone to allometric interference. The PCA scores were tested for outliers by examining two-dimensional plots of the scores with the verification that no serious nonlinearity existed. The PCA scores of the characters from all the principle component axes other than the first were then entered into a canonical discriminant analysis.

RESULTS

Univariate analysis:

The univariate analysis indicates that there is variability in the majority of the character means when compared between collection sites. Appendices A and B provide per collection site the sample size, mean, standard error of mean, minimum data entry, and maximum data entry of each character for the respective male and female data. Tables 2 and 3 display the ANOVA results for the respective male and female data. Post hoc power calculations (Lenth, 2006) resulted in over 80% power to detect a difference of 0.8 within 1 standard deviation between groups with a 0.05 level of significance. For the males, 33 of 54 (61.1%) characters were statistically significant at the alpha 0.05 level while for the females, 41 of 49 (83.7%) characters were. Multiple comparisons of the character averages using the Bonferroni correction was conducted to determine which collection sites displayed significant mean differences at the alpha 0.05 level. Appendices C and D provide the multiple comparisons of the male and female data respectively data using the Bonferroni correction. A larger wing width and a longer radial 5 vein length discriminated the male Patuxent NWRR specimens from all other collection sites. Shorter tibia length discriminated the male Ossabaw Island sand flies from males of the other four sites. In the female data, two characters were discriminative: longer femurs and longer spermathecae separated the Fort Bragg females from female flies collected at other sites.

The phenograms based upon the mean relationships of the significant characters are shown in Figure 2 for the males and Figure 3 for the females. The same analyses were also conducted for all variables and, as expected, the resulting phenograms (not shown)

were nearly identical to the respective Figures 2 and 3 based just on characters found to be significant. Unlike in Dias et al. (1999) using the *Lu. whitmani* model, the phenograms produced fundamentally different depictions of similarity among the collected flies based on gender.

Multivariate analysis:

The results of the multivariate analyses indicate some divergence between the populations although complete separation is never attained. Separation among the groups was enhanced by using all the data (independent procedure) versus the step-wise procedure where characters of low information content were eliminated. Common logarithmic transformation of the character measurements gave virtually the same classification results. Discriminant analysis of the ratio data, both actual and log-transformed, did not show any significant separation by collection site. Discriminant analysis of the ANOVA significant characters, both actual and log-transformed did not show any significant separation by collection site.

In the male data, the step-wise statistical method (using the Mahalanobis distance and criteria of F value entry of 3.84 and removal value of 2.71) of the CDA eliminated 44 characters of low information content until a subset of ten informative characters remained. The ten most informative, arranged in decreasing order of significance, were: palp III length, flagellomere V ratio, tibia length, wing width, gonostylus width, tibia, femur, gonocoxite width, palp II length, palp I ratio, and flagellomere VI width. Wing width and tibia length were previously found to be of a discriminative nature in the multiple comparison results of the univariate analysis. Tests of significance for the model as a whole indicated that the model functioned and was discriminating: the Wilks'

lambda was significant for all four discriminant functions and the Wilks' lambda difference tests showed classification better than chance. The canonical correlations for the first and second functions in the canonical discriminant analysis were .646 and .555 respectively. Cumulative percent of variance for the first and second axes was 69.8%; four canonical axes were used with cumulative percent of third axis being 91.6%. Only 59.5% of original grouped cases were correctly classified by the model generated by the analysis. Cross-validated grouped cases yielded only 48.5% of cases correctly classified. If the model was changed from step-wise procedure to independently entered data, the original grouped cases correctly classified increased to 76.0% but the cross-validated grouped cases correctly classified declined to 36%. Figure 4 is the CDA scatter plot of all the analyzed character measurements (independent procedure) of the male sand flies.

In the female data, the step-wise statistical method (using the Mahalanobis distance and criteria of F value entry of 3.84 and removal value of 2.71) of the CDA eliminated 42 characters of low information content until a subset of 7 characters remained. The seven most informative, arranged in decreasing order of significance, were: femur length, palp I length, flagellomere V length, wing width, labrum length, palp II width, and spermathecae length. The prior univariate multiple comparison results had also indicated discriminative properties of femur length and spermathecae length in females. Tests of significance for the model as a whole indicated that the model functioned and was discriminating: the Wilks' lambda was significant for four of the five discriminant functions and the Wilks' lambda difference tests showed classification better than chance. The canonical correlations for the first and second functions in the canonical discriminant analysis were .759 and .619 respectively. Cumulative percent of variance for the first

and second axes was 79.4%; five canonical axes were used with the third axis accounting for a cumulative 95.7% of the explained variation. Only 57.1% of original grouped cases were correctly classified by the model generated by the analysis. Cross-validated grouped cases yielded only 47.1% of cases correctly classified. If the model was changed from step-wise procedure to independently entered data, the original grouped cases correctly classified did increase to 78.8% but the cross-validated grouped cases correctly classified improved only marginally to 59.6%. Figure 5 is the CDA scatter plot of all the analyzed character measurements (independent procedure) of the female sand flies.

An assumption of discriminant analysis is the equality of population dispersion matrices (homoscedasticity) although the method is usually robust enough to overcome even this transgression if it is not too serious of a violation (Pimentel, 1992). In order to eliminate any major heterescedasticity, CDA was conducted on each gender without using any ratios, width measurements, or any character that had a significant Levene Statistic in the test of homogeneity of variances. The ratios and widths were not used since these measurements generally displayed the greater variances when examined in standardized residual plots. In the males, aside from the ratios and widths, the following characters were excluded: palp2len, flag2len, and r5l. As shown in Figure 6, the result of the CDA still could not separate the male sand flies according to collection site. For the females the following characters were excluded in addition to the ratios and widths: hdlength, flaglen, r5l, alpha, and tibia. As in the males, the CDA did not separate the collections sites (Figure 7).

The “size-free” analysis modeled after Dujardin et al. (2004) did not enhance separation among the collection sites as compared to the general discriminant analyses of

above. The characters that displayed an orthogonal projection to the first principle component axis were found to be marginally discriminative when entered into a CDA via the independent procedure. In the male data, tests of significance for the model as a whole indicated that the model functioned and was discriminating: the Wilks' lambda was significant for the first three discriminant functions and the Wilks' lambda difference tests showed classification better than chance. The canonical correlations for the first and second functions in the canonical discriminant analysis were .628 and .557 respectively. Cumulative percent of variance for the first and second axes was 78.2%; four canonical axes were used with cumulative percent of third axis being 94.7%. Original grouped cases were correctly classified by the model 61.0% of the time while cross-validated grouped cases yielded only 43.5% of cases correctly classified. Figure 8 is the CDA scatter plot of the male PCA scores for all axes except the first.

In the female data, the results of entering all the PCA scores except from the first principle component were nearly the same as for the males. Tests of significance for the model as a whole indicated that the model functioned and was discriminating: the Wilks' lambda was significant for all four discriminant functions and the Wilks' lambda difference tests showed classification better than chance. The canonical correlations for the first and second functions in the canonical discriminant analysis were .698 and .662 respectively. Cumulative percent of variance for the first and second axes was 68.7%; five canonical axes were used with cumulative percent of the fourth axis being 95.7%. Original grouped cases were correctly classified by the model 67.1% of the time while cross-validated grouped cases yielded 55.4% of cases correctly classified. Figure 9 is the CDA scatter plot of the female PCA scores for all axes except the first.

DISCUSSION

Variation is ubiquitous in nature as Darwin (1859) pointed out with "No one supposes that all individuals of the same species are cast in the same actual mould." It falls to statistical methodology to supply information that will serve as the basis to make the decision of how much variation should be allowed before the population is considered fundamentally different from other populations. ANOVA is used to estimate and test hypotheses regarding population variances and means (Daniel, 1999). In this study, ANOVA tested the null hypothesis that all *Lu. shannoni* collected from different sites have equal means of the characters examined against the alternative hypothesis that at least one collection site shows a different character mean when compared to the others. Clearly, the results indicate that the populations display variation as evidenced by more than half the characters in each gender being statistically significant at the alpha 0.05 level and five being discriminative. Dias et al. (1999) also found over half the characters significant and six discriminative in the analysis of *Lu. whitmani* but were very careful to keep within the limits of the ANOVA methodology when examining geographical variation. No promulgation of a cryptic species was made because the authors stated that the results are simply not sufficient to determine taxonomic status but to only indicate possible biogeographical populations.

The phenograms (Figures 2 and 3) depict the similarity among the grouped specimens captured from each collecting site based on the means of the significant characters found in the ANOVA analysis. Little information can be derived when viewing these phenograms as separate entities. The fact that the parameters used to draw

these diagrams were significantly different to begin with precludes the phenogram from displaying an absence of divergence among the groups (the null hypothesis). The true value of these phenograms will be in the comparison with other character analyses such as the molecular studies of the ITS2 and the CO1 sequences. If consistency of groupings is found throughout different studies that are similar to these phenograms, then there will exist multiple results indicating that the populations are at least heterogeneous with some displaying more similarity to certain populations than others.

A complicating result in this study is that the male and female phenograms did not produce the same clustering irrespective of gender such as Dias (1999) found for *Lu. whitmani*. The hierarchical cluster analysis of the female data was re-run with the exclusion of the Suwannee NWR flies (results not shown) since the male data did not include this collection site. There was no fundamental difference in the subsequent phenogram; the Suwannee branch no longer existed yet all other groupings remained intact. The inclusion of the Suwannee NWR flies in the female data did not skew the clustering relationship to the point where its exclusion would produce comparable clustering to the males. The argument can be made that when the groups cluster the same irrespective of gender, it is due to the simple size variation manifested in both sexes of the groups. However, if physical modifications are being selected for in one gender in a particular population then this may be the incipient stage of reproductive isolation as this gender may become so dissimilar to other populations that breeding becomes restricted. This scenario may have happened with the sibling species of the *Verrucarum* species group of *Lutzomyia* where the females are nearly indistinguishable morphologically yet the males can readily be identified to species. The same holds true for the sympatric

species of *Lu. shannoni, Lu. abonnenci* and *Lu. pestanai* where the females cannot be differentiated, yet the genital morphology readily separates the males (Young and Duncan, 1994). The counter argument to this speculation is that all of the flies from each collection site are homogenous and are separated on an arbitrary basis of geography; the observed differences in clustering on the respective phenograms is simply a manifestation of the use of different characters and sexual dimorphism.

The CDAs of the character measurements also indicates that there is some degree of divergence, albeit relatively minor, between the sand fly groups collected at each collection site. In both genders, over 75% of original grouped cases could be correctly classified from the in-group data pool although neither sex obtained over 60% of cases correctly classified in cross-validation. Claridge and Gillham (1992) stated that if populations could not be separated completely in a CDA, then they have to be viewed as morphologically inseparable at least on the basis of the characters examined. Clearly, the grouped sand flies of the collection sites could not be "separated completely" as the percent correctly classified in the cross-validation classifications hovered in the high fifty percent range. This figure is especially paltry when one realizes that by chance alone there is a 1 in 5 (5 collection sites) probability of placing the males in the correct classification and a 1 in 6 (6 collection sites) probability for the females.

The size-free analysis using the principle component scores did not enhance separation. Size may still be a confounding factor, although it is not possible to make a determination in this study. The initial CDAs did not separate the populations and the subsequent decrease in variation by removal of the first principle component axes in the PCAs of each collecting site did not produce fully separated populations. If there is size

confounding, it would appear to be minor as the CDAs from the PCA scores with the first axis removed were quite similar to the initial CDAs. Pimentel (1992) stated one of the assumptions of PCA is that the variables exist in the form of a straight line but this often fails to test true as an individual specimen may exhibit different dimensions of a character (different allometric vectors) when compared to others of the same population. This allometry is inherently nonlinear and while it is technically a violation of the PCA assumptions, the technique is usually robust enough to overcome the transgression. Outliers and nonlinearity of the PCA scores were checked and found to be acceptable. In addition, when the ratios of length/width were computed in the CDAs, a separation well below the character data resulted indicating that there was not a serious difference in allometric size. While certain ratios were statistically significant at the 0.05 alpha level in the univariate analysis, the ratios as a group exhibited very poor discriminant properties.

What variation due to size exists in the data may be attributed to collecting at different times of the year among the collection sites. Sand flies collected during the spring were used in samples for Fort Rucker, Suwannee NWR, and Patuxent NWRR. All other sand flies were collected during the late summer/early fall season. An inverse relationship has been reported for the effects of temperature on adult size in mosquitoes (Clements 2000; Tun-lin et al, 2000). If this relationship holds true for sand flies, then there is the possibility that larger sand flies of Fort Rucker, Suwannee NWR, and Patuxent NWRR were compared to the smaller flies of Fort Bragg, Fort Campbell, and Ossabaw Island. The reasoning behind this is that sand flies collected in the spring could be larger due to larval development in the cooler months of the preceding fall prior to

diapause versus the sand flies collected in the fall when larval development would have occurred during the warmer summer months.

Combining the univariate and multivariate analyses, the conclusion is the following: Among the characters used in this study, there is variation between the collection sites but it is not of a sufficient degree of difference to enable one to discriminate the collection sites. This indicates that the specimens from each collection site are within the normal variance of the species, i.e., there exists one population in the southeast U.S. with adequate gene flow to maintain homogeneity. Yet, it is important to keep in mind that these two analyses, univariate and multivariate, are on one type of character: morphological. Standing alone it is just an indication, but standing with other character analyses such as molecular, it can form the basis for a definitive step towards taxonomic determination.

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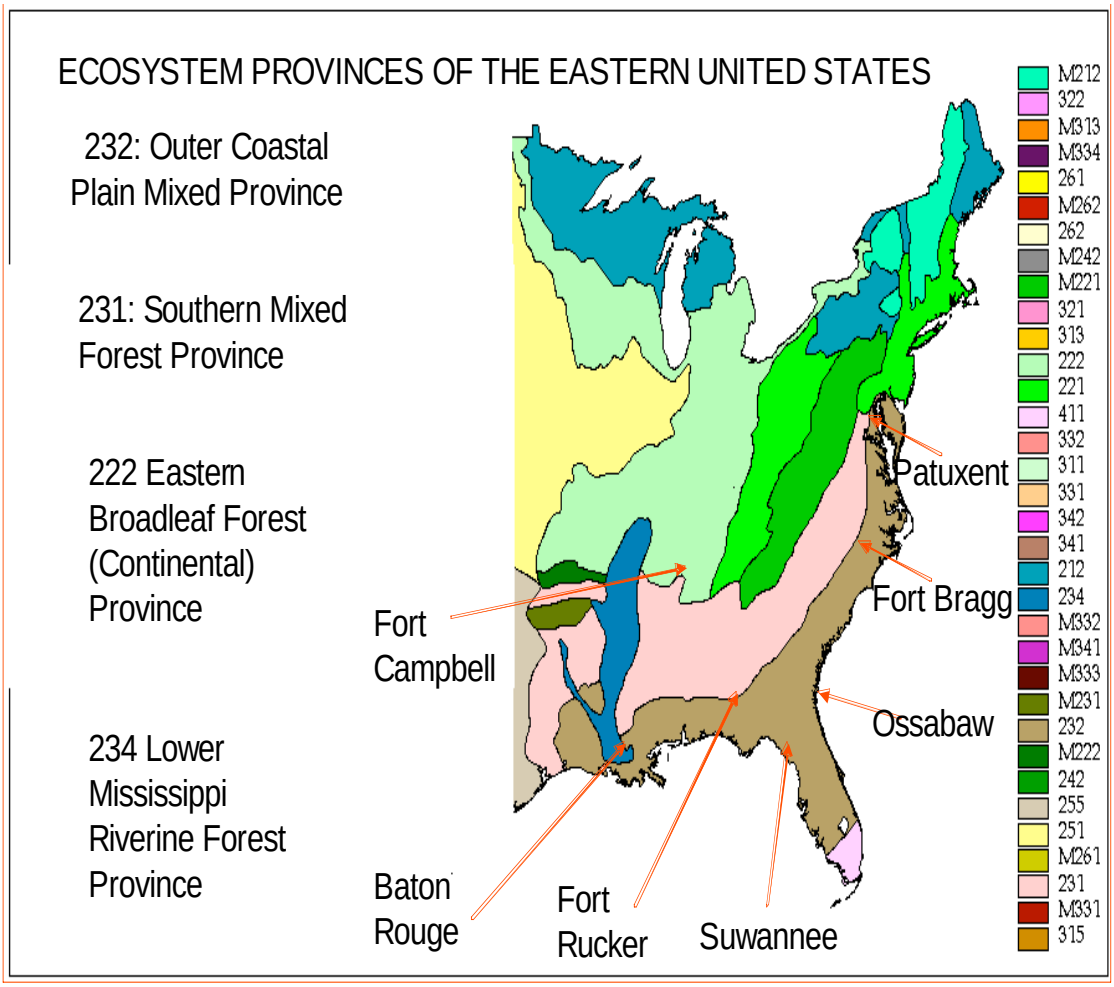

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Figure 1. Location of collecting sites in relation to designated USDA ecosystem provinces.

Table 1. List of morphological characters, abbreviations used, gender applied to, and description of measurement

Character	**Abbreviation of character used in statistical analyses**	**Gender for which character used**	**Description of character measurement**
Head length	HDLENGTH	Both	Measurement from vertex to ventral edge of clypeus
Clypeus	CLYPEUS	Both	Measurement from subgenal suture to edge of clypeus
Interocular	INTEROC	Both	Smallest measurement between compound eyes
Labrum length	LABRUML	Both	Measurement from edge of clypeus to apical tip of labrum
Labrum width	LABRUMW	Both *	Widest measurement of labrum width
Labrum ratio	LABRRAT	Both *	Ration of length/width of labrum
Palpal segment I length	PALP1LEN	Both	Measurement from basal articulation to fusion line with palp II
Palpal segment I width	PALP1WID	Both	Widest measurement of palpal I segment width
Palpal segment I ratio	PALP1RAT	Both	Ratio of length/width of palpal I segment
Palpal segment II length	PALP2LEN	Both	Measurement of palpal II segment length
Palpal segment II width	PALP2WID	Both	Widest measurement of palpal II segment width
Palpal segment II ratio	PALP2RAT	Both	Ratio of length/width of palpal II segment
Palpal segment III length	PALP3LEN	Both	Measurement of palpal III segment length
Palpal segment III width	PALP3WID	Both	Widest measurement of palpal III segment width
Palpal segment III ratio	PALP3RAT	Both	Ratio of length/width of palpal III segment
Palpal segment IV length	PALP4LEN	Both	Measurement of palpal IV segment length
Palpal segment IV width	PALP4WID	Both	Widest measurement of palpal IV width
Palpal segment IV ratio	PALP4RAT	Both	Ratio of length/width of palpal IV segment
Palpal segment V length	PALP5LEN	Both *	Measurement of palpal V segment length
Palpal segment V width	PALP5WID	Both *	Widest measurement of palpal V width
Palpal segment V ratio	PALP5RAT	Both *	Ratio of length/width of palpal V segment
Flagellomere I length	FLAG1LEN	Both	Measurement of flagellomere I length
Flagellomere I width	FLAG1WID	Both	Widest measurement of flagellomere I width
Flagellomere I ratio	FLAG1RAT	Both	Ratio of length/width of flagellomere I segment
Flagellomere II length	FLAG2LEN	Both	Measurement of flagellomere II length
Flagellomere II width	FLAG2WID	Both	Widest measurement of flagellomere II width
Flagellomere II ratio	FLAG2RAT	Both	Ratio of length/width of flagellomere II segment
Flagellomere III length	FLAG3LEN	Both	Measurement of flagellomere III length
Flagellomere III width	FLAG3WID	Both	Widest measurement of flagellomere III width
Flagellomere III ratio	FLAG3RAT	Both	Ratio of length/width of flagellomere III segment
Flagellomere IV length	FLAG4LEN	Both	Measurement of flagellomere IV length
Flagellomere IV width	FLAG4WID	Both	Widest measurement of flagellomere IV width
Flagellomere IV ratio	FLAG4RAT	Both	Ratio of length/width of flagellomere IV segment
Flagellomere V length	FLAG5LEN	Both	Measurement of flagellomere V length
Flagellomere V width	FLAG5WID	Both	Widest measurement of flagellomere V width
Flagellomere V ratio	FLAG5RAT	Both	Ratio of length/width of flagellomere V segment
Flagellomere VI length	FLAG6LEN	Both	Measurement of flagellomere VI length
Flagellomere VI width	FLAG6WID	Both	Widest measurement of flagellomere VI width
Flagellomere VI ratio	FLAG6RAT	Both	Ratio of length/width of flagellomere VI segment
Flagellomere VII length	FLAG7LEN	Both	Measurement of flagellomere VII length
TABLE 1 CONTINUED			

Table 1. List of morphological characters, abbreviations used, gender applied to, and description of measurement (continued)

Character	Abbreviation of character used in statistical analyses	Gender for which character used	Description of character measurement
Flagellomere VII width	FLAG7WID	Both	Widest measurement of flagellomere VII width
Flagellomere VII ratio	FLAG7RAT	Both	Ratio of length/width of flagellomere VII segment
Flagellomere VIII length	FLAG8LEN	Both *	Measurement of flagellomere VIII length
Flagellomere VIII width	FLAG8WID	Both *	Widest measurement of flagellomere VIII width
Flagellomere VIII ratio	FLAG8RAT	Both *	Ratio of length/width of flagellomere VIII segment
Flagellomere IX length	FLAG9LEN	Both *	Measurement of flagellomere IX length
Flagellomere IX width	FLAG9WID	Both *	Widest measurement of flagellomere IX width
Flagellomere IX ratio	FLAG9RAT	Both *	Ratio of length/width of flagellomere IX segment
Flagellomere X length	FLAG10L	Both *	Measurement of flagellomere X length
Flagellomere X width	FLAG10W	Both *	Widest measurement of flagellomere X width
Flagellomere X ratio	FLAG10RA	Both *	Ratio of length/width of flagellomere X segment
R-5 length	R5L	Both	Length of wing vein R5
Wing width	WINGWID	Both	Measurement of widest width of wing
Alpha length	ALPHA	Both	Length of wing vein R2 from its junction with R3 to the costa
Delta length	DELTA	Both	Length of wing vein R1 that extends beyond junction of R2 and R3
Beta length	BETA	Both	Length of wing vein R from junction of R2 and R3 to junction with R4
Gamma length	GAMMA	Both	Length of wing vein R from origin of R5 to origin of R2+3 and R4
Femur length	FEMUR	Both	Length of femur
Tibia length	TIBIA	Both	Length of tibia
Cercus length	CERCUSL	Female only	Measurement of widest width of cercus
Spermathecae length	SPERML	Female only	Length of spermathecae
Spermathecae width	SPERMW	Female only	Measurement of widest width of spermathecae
Spermathecae ratio	SPERMRAT	Female only	Ratio of length/width of spermathecae
Gonostylus length	GSTYLLEN	Male only	Length of gonostylus
Gonostylus width	GSTYLWID	Male only	Measurement of widest width of gonostylus
Gonostylus ratio	GSTYLRAT	Male only	Ratio of length/width of gonostylus
Gonocoxite length	GCXLEN	Male only	Length of gonocoxite
Gonocoxite width	GCXWID	Male only	Measurement of widest width of gonocoxite
Gonocoxite ratio	GCXRAT	Male only	Ratio of length/width of gonocoxite
Lateral lobe length	LATLBL	Male only	Length of lateral lobe
Lateral lobe width	LATLBW	Male only	Measurement of widest width of lateral lobe
Lateral lobe ration	LATLBRAT	Male only	Ratio of length/width oflateral lobe

* Designates characters that were excluded from the analyses due to having exceeded more than 10% of the total number with blank values

Table 2. ANOVA results of the male *Lu. shannoni* data

ANOVA						
Character	Comparison	Sum of Squares	df	Mean Square	F	Sig.
hdlength	Between Groups	2,275.255	4	568.814	0.937	0.444
	Within Groups	109,933.885	181	607.370		
	Total	112,209.140	185			
clypeus	Between Groups	900.095	4	225.024	2.572	0.039
	Within Groups	16,975.669	194	87.503		
	Total	17,875.764	198			
interoc	Between Groups	453.813	4	113.453	1.871	0.117
	Within Groups	11,457.780	189	60.623		
	Total	11,911.593	193			
labruml	Between Groups	2,873.647	4	718.412	1.478	0.210
	Within Groups	94,317.308	194	486.172		
	Total	97,190.955	198			
palp1len	Between Groups	430.161	4	107.540	4.155	0.003
	Within Groups	5,047.439	195	25.884		
	Total	5,477.600	199			
palp1wid	Between Groups	97.352	4	24.338	2.465	0.046
	Within Groups	1,925.395	195	9.874		
	Total	2,022.747	199			
palp1rat	Between Groups	0.852	4	0.213	2.923	0.022
	Within Groups	14.204	195	0.073		
	Total	15.056	199			
palp2len	Between Groups	2,083.472	4	520.868	8.344	0.000
	Within Groups	12,172.528	195	62.423		
	Total	14,256.000	199			
palp2wid	Between Groups	108.489	4	27.122	3.775	0.006
	Within Groups	1,400.920	195	7.184		
	Total	1,509.410	199			
palp2rat	Between Groups	1.371	4	0.343	0.893	0.469
	Within Groups	74.868	195	0.384		
	Total	76.238	199			
palp3len	Between Groups	283.905	4	70.976	1.096	0.360
	Within Groups	12,623.850	195	64.738		
	Total	12,907.755	199			
palp3wid	Between Groups	209.334	4	52.334	5.229	0.001
	Within Groups	1,951.720	195	10.009		
	Total	2,161.055	199			
palp3rat	Between Groups	6.783	4	1.696	4.256	0.003
	Within Groups	77.691	195	0.398		
	Total	84.474	199			
palp4len	Between Groups	118.311	4	29.578	1.132	0.343
	Within Groups	5,093.634	195	26.121		
	Total	5,211.945	199			

Continued

Table 2. ANOVA results of the male *Lu. shannoni* data (cont.)

Character	Comparison	Sum of Squares	df	Mean Square	F	Sig.
palp4wid	Between Groups	54.827	4	13.707	2.299	0.060
	Within Groups	1,162.603	195	5.962		
	Total	1,217.430	199			
palp4rat	Between Groups	1.604	4	0.401	1.937	0.106
	Within Groups	40.380	195	0.207		
	Total	41.984	199			
flag1len	Between Groups	4,508.000	4	1,127.000	2.129	0.079
	Within Groups	103,220.000	195	529.333		
	Total	107,728.000	199			
flag1wid	Between Groups	57.307	4	14.327	1.502	0.203
	Within Groups	1,860.342	195	9.540		
	Total	1,917.650	199			
flag1rat	Between Groups	55.185	4	13.796	3.218	0.014
	Within Groups	836.061	195	4.287		
	Total	891.246	199			
flag2len	Between Groups	1,034.759	4	258.690	5.161	0.001
	Within Groups	9,674.493	193	50.127		
	Total	10,709.253	197			
flag2wid	Between Groups	14.026	4	3.506	0.660	0.621
	Within Groups	1,025.909	193	5.316		
	Total	1,039.935	197			
flag2rat	Between Groups	5.205	4	1.301	1.729	0.145
	Within Groups	145.251	193	0.753		
	Total	150.456	197			
flag3len	Between Groups	409.727	4	102.432	1.683	0.156
	Within Groups	11,687.284	192	60.871		
	Total	12,097.011	196			
flag3wid	Between Groups	34.699	4	8.675	1.380	0.242
	Within Groups	1,206.616	192	6.284		
	Total	1,241.315	196			
flag3rat	Between Groups	5.249	4	1.312	1.815	0.128
	Within Groups	138.074	191	0.723		
	Total	143.323	195			
flag4len	Between Groups	449.170	4	112.293	2.520	0.043
	Within Groups	8,511.455	191	44.563		
	Total	8,960.625	195			
flag4wid	Between Groups	58.546	4	14.636	3.311	0.012
	Within Groups	835.403	189	4.420		
	Total	893.949	193			
flag4rat	Between Groups	9.830	4	2.457	3.531	0.008
	Within Groups	131.519	189	0.696		
	Total	141.349	193			

Continued

Table 2. ANOVA results of the male *Lu. shannoni* data (cont.)

Character	Comparison	Sum of Squares	df	Mean Square	F	Sig.
flag5len	Between Groups	560.431	4	140.108	3.286	0.012
	Within Groups	8,144.873	191	42.643		
	Total	8,705.305	195			
flag5wid	Between Groups	93.872	4	23.468	6.194	0.000
	Within Groups	723.670	191	3.789		
	Total	817.542	195			
flag5rat	Between Groups	14.953	4	3.738	5.922	0.000
	Within Groups	120.573	191	0.631		
	Total	135.526	195			
flag6len	Between Groups	577.166	4	144.292	3.404	0.010
	Within Groups	8,010.530	189	42.384		
	Total	8,587.696	193			
flag6wid	Between Groups	104.409	4	26.102	6.209	0.000
	Within Groups	786.169	187	4.204		
	Total	890.578	191			
flag6rat	Between Groups	9.447	4	2.362	3.022	0.019
	Within Groups	146.163	187	0.782		
	Total	155.611	191			
flag7len	Between Groups	508.961	4	127.240	2.499	0.044
	Within Groups	9,267.295	182	50.919		
	Total	9,776.256	186			
flag7wid	Between Groups	83.070	4	20.768	4.992	0.001
	Within Groups	757.197	182	4.160		
	Total	840.267	186			
flag7rat	Between Groups	14.310	4	3.577	4.879	0.001
	Within Groups	133.458	182	0.733		
	Total	147.768	186			
r5l	Between Groups	104,862.578	4	26,215.644	8.571	0.000
	Within Groups	571,988.735	187	3,058.763		
	Total	676,851.313	191			
wingwid	Between Groups	40,861.401	4	10,215.350	10.976	0.000
	Within Groups	173,102.997	186	930.661		
	Total	213,964.398	190			
alpha	Between Groups	74,987.165	4	18,746.791	8.463	0.000
	Within Groups	414,212.314	187	2,215.039		
	Total	489,199.479	191			
delta	Between Groups	24,131.001	4	6,032.750	3.899	0.005
	Within Groups	290,912.523	188	1,547.407		
	Total	315,043.523	192			
beta	Between Groups	6,572.270	4	1,643.068	1.638	0.166
	Within Groups	189,527.214	189	1,002.789		
	Total	196,099.485	193			

Continued

Table 2. ANOVA results of the male *Lu. shannoni* data (cont.)

Character	Comparison	Sum of Squares	df	Mean Square	F	Sig.
gamma	Between Groups	8,366.680	4	2,091.670	2.537	0.042
	Within Groups	155,845.691	189	824.580		
	Total	164,212.371	193			
femur	Between Groups	122,467.355	4	30,616.839	8.638	0.000
	Within Groups	638,003.910	180	3,544.466		
	Total	760,471.265	184			
tibia	Between Groups	463,304.144	4	115,826.036	10.233	0.000
	Within Groups	2,116,567.106	187	11,318.541		
	Total	2,579,871.250	191			
gstyllen	Between Groups	716.496	4	179.124	2.229	0.067
	Within Groups	15,667.678	195	80.347		
	Total	16,384.174	199			
gstylwid	Between Groups	1,161.338	4	290.334	6.970	0.000
	Within Groups	8,122.655	195	41.655		
	Total	9,283.992	199			
gstylrat	Between Groups	16.677	4	4.169	7.156	0.000
	Within Groups	113.607	195	0.583		
	Total	130.284	199			
gcxlen	Between Groups	9,230.000	4	2,307.500	4.624	0.001
	Within Groups	97,320.000	195	499.077		
	Total	106,550.000	199			
gcxwid	Between Groups	527.000	4	131.750	2.173	0.074
	Within Groups	11,825.000	195	60.641		
	Total	12,352.000	199			
gcxrat	Between Groups	1.426	4	0.356	2.539	0.041
	Within Groups	27.379	195	0.140		
	Total	28.805	199			
latlbl	Between Groups	1,413.000	4	353.250	0.763	0.551
	Within Groups	90,305.000	195	463.103		
	Total	91,718.000	199			
latlbw	Between Groups	286.183	4	71.546	2.855	0.025
	Within Groups	4,886.072	195	25.057		
	Total	5,172.255	199			
latlbrat	Between Groups	31.104	4	7.776	3.422	0.010
	Within Groups	443.071	195	2.272		
	Total	474.175	199			

End Table 2

Table 3. ANOVA results of the female *Lu. shannoni* data

ANOVA

Character	Comparison	Sum of Squares	df	Mean Square	F	Sig.
hdlength	Between Groups	15875.555	5	3175.111	5.390	.000
	Within Groups	132553.883	225	589.128		
	Total	148429.437	230			
clypeus	Between Groups	3586.007	5	717.201	7.221	.000
	Within Groups	23142.849	233	99.326		
	Total	26728.856	238			
interoc	Between Groups	2505.378	5	501.076	6.222	.000
	Within Groups	18604.136	231	80.537		
	Total	21109.514	236			
labruml	Between Groups	15221.668	5	3044.334	8.271	.000
	Within Groups	85763.269	233	368.083		
	Total	100984.937	238			
palp1len	Between Groups	2304.049	5	460.810	12.292	.000
	Within Groups	8772.680	234	37.490		
	Total	11076.729	239			
palp1wid	Between Groups	356.759	5	71.352	4.686	.000
	Within Groups	3563.114	234	15.227		
	Total	3919.873	239			
palp1rat	Between Groups	2.112	5	.422	4.733	.000
	Within Groups	20.883	234	.089		
	Total	22.995	239			
palp2len	Between Groups	1046.340	5	209.268	2.621	.025
	Within Groups	18443.431	231	79.842		
	Total	19489.771	236			
palp2wid	Between Groups	606.706	5	121.341	8.777	.000
	Within Groups	3207.441	232	13.825		
	Total	3814.147	237			
palp2rat	Between Groups	20.000	5	4.000	7.358	.000
	Within Groups	125.572	231	.544		
	Total	145.572	236			
palp3len	Between Groups	2090.791	5	418.158	4.671	.000
	Within Groups	20411.481	228	89.524		
	Total	22502.272	233			
palp3wid	Between Groups	741.215	5	148.243	6.452	.000
	Within Groups	5261.833	229	22.977		
	Total	6003.048	234			

Continued

Table 3. ANOVA results of the female *Lu. shannoni* data (cont.)

Character	Comparison	Sum of Squares	df	Mean Square	F	Sig.
palp3rat	Between Groups	15.454	5	3.091	6.042	.000
	Within Groups	116.628	228	.512		
	Total	132.082	233			
palp4len	Between Groups	450.251	5	90.050	2.545	.029
	Within Groups	7997.817	226	35.389		
	Total	8448.069	231			
palp4wid	Between Groups	117.067	5	23.413	2.648	.024
	Within Groups	1998.338	226	8.842		
	Total	2115.405	231			
palp4rat	Between Groups	3.775	5	.755	3.016	.012
	Within Groups	56.564	226	.250		
	Total	60.339	231			
flag1len	Between Groups	5090.000	5	1018.000	2.139	.062
	Within Groups	111350.000	234	475.855		
	Total	116440.000	239			
flag1wid	Between Groups	390.234	5	78.047	5.147	.000
	Within Groups	3548.306	234	15.164		
	Total	3938.541	239			
flag1rat	Between Groups	88.823	5	17.765	4.526	.001
	Within Groups	918.416	234	3.925		
	Total	1007.239	239			
flag2len	Between Groups	720.562	5	144.112	3.063	.011
	Within Groups	11010.938	234	47.055		
	Total	11731.500	239			
flag2wid	Between Groups	73.765	5	14.753	2.266	.049
	Within Groups	1523.433	234	6.510		
	Total	1597.198	239			
flag2rat	Between Groups	5.481	5	1.096	2.477	.033
	Within Groups	103.580	234	.443		
	Total	109.061	239			
flag3len	Between Groups	1142.775	5	228.555	4.607	.000
	Within Groups	11609.325	234	49.613		
	Total	12752.100	239			
flag3wid	Between Groups	100.153	5	20.031	3.967	.002
	Within Groups	1181.588	234	5.050		
	Total	1281.741	239			
flag3rat	Between Groups	7.701	5	1.540	3.539	.004
	Within Groups	101.848	234	.435		
	Total	109.550	239			

Continued

Table 3. ANOVA results of the female *Lu. shannoni* data (cont.)

Character	Comparison	Sum of Squares	df	Mean Square	F	Sig.
flag4len	Between Groups	1148.618	5	229.724	5.350	.000
	Within Groups	10047.923	234	42.940		
	Total	11196.541	239			
flag4wid	Between Groups	39.150	5	7.830	1.628	.153
	Within Groups	1125.141	234	4.808		
	Total	1164.291	239			
flag4rat	Between Groups	4.813	5	.963	2.234	.052
	Within Groups	100.813	234	.431		
	Total	105.626	239			
flag5len	Between Groups	1590.493	5	318.099	7.361	.000
	Within Groups	10068.423	233	43.212		
	Total	11658.916	238			
flag5wid	Between Groups	27.681	5	5.536	1.136	.342
	Within Groups	1135.762	233	4.875		
	Total	1163.443	238			
flag5rat	Between Groups	3.271	5	.654	1.488	.194
	Within Groups	102.417	233	.440		
	Total	105.689	238			
flag6len	Between Groups	2488.977	5	497.795	9.389	.000
	Within Groups	12299.840	232	53.017		
	Total	14788.817	237			
flag6wid	Between Groups	84.388	5	16.878	3.703	.003
	Within Groups	1061.983	233	4.558		
	Total	1146.370	238			
flag6rat	Between Groups	11.765	5	2.353	4.646	.000
	Within Groups	117.489	232	.506		
	Total	129.254	237			
flag7len	Between Groups	1739.330	5	347.866	7.465	.000
	Within Groups	9878.527	212	46.597		
	Total	11617.857	217			
flag7wid	Between Groups	53.899	5	10.780	2.449	.035
	Within Groups	933.080	212	4.401		
	Total	986.978	217			
flag7rat	Between Groups	11.292	5	2.258	4.502	.001
	Within Groups	106.343	212	.502		
	Total	117.635	217			
r5l	Between Groups	278261.031	5	55652.206	9.005	.000
	Within Groups	1421382.901	230	6179.926		
	Total	1699643.932	235			
wingwid	Between Groups	111913.565	5	22382.713	12.464	.000
	Within Groups	409440.281	228	1795.791		
	Total	521353.846	233			

Continued

Table 3. ANOVA results of the female *Lu. shannoni* data (cont.)

Character	Comparison	Sum of Squares	df	Mean Square	F	Sig.
alpha	Between Groups	95105.210	5	19021.042	6.083	.000
	Within Groups	719211.316	230	3127.006		
	Total	814316.525	235			
delta	Between Groups	12793.272	5	2558.654	1.211	.305
	Within Groups	485837.237	230	2112.336		
	Total	498630.508	235			
beta	Between Groups	7980.427	5	1596.085	1.640	.150
	Within Groups	224850.796	231	973.380		
	Total	232831.224	236			
gamma	Between Groups	32636.453	5	6527.291	5.533	.000
	Within Groups	272521.353	231	1179.746		
	Total	305157.806	236			
femur	Between Groups	225067.278	5	45013.456	15.842	.000
	Within Groups	619443.579	218	2841.484		
	Total	844510.857	223			
tibia	Between Groups	703851.226	5	140770.245	9.677	.000
	Within Groups	3258360.061	224	14546.250		
	Total	3962211.287	229			
cercusl	Between Groups	7356.269	5	1471.254	4.116	.001
	Within Groups	82572.101	231	357.455		
	Total	89928.370	236			
sperml	Between Groups	2530.531	5	506.106	13.044	.000
	Within Groups	8187.084	211	38.801		
	Total	10717.616	216			
spermw	Between Groups	64.130	5	12.826	1.220	.301
	Within Groups	2219.127	211	10.517		
	Total	2283.257	216			
spermrat	Between Groups	4.269	5	.854	4.456	.001
	Within Groups	40.426	211	.192		
	Total	44.695	216			

End Table 3

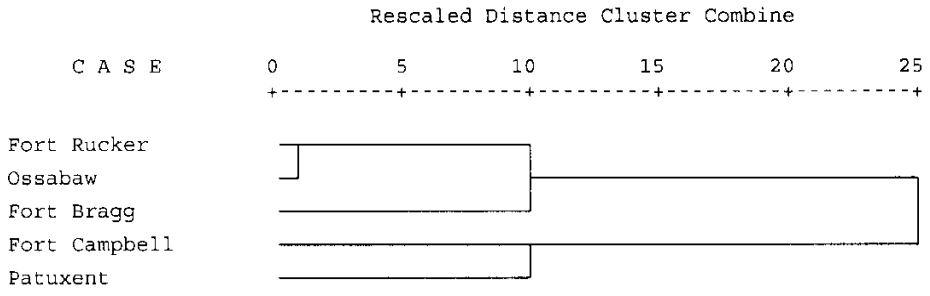

Figure 2. Phenogram of male binary data of characters displaying significant means at the 0.05 alpha level in a hierarchical cluster analysis [average linkage (between groups) by simple matching method]. Values of the distance coefficients at each step are shown with actual distances rescaled to numbers between 0 and 25, preserving the ratio of the distances between steps. Connected vertical lines designate joined cases.

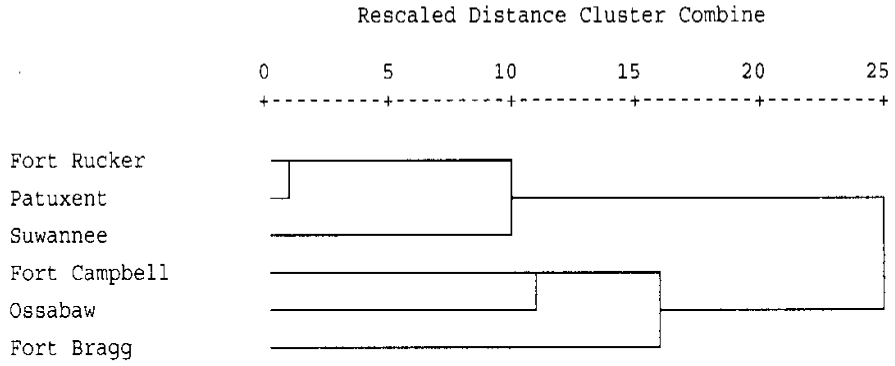

Figure 3. Phenogram of female binary data of characters displaying significant means at the 0.05 alpha level in hierarchical cluster analysis [average linkage (between groups) by simple matching method]. Values of the distance coefficients at each step are shown with actual distances rescaled to numbers between 0 and 25, preserving the ratio of the distances between steps. Connected vertical lines designate joined cases.

Figure 4. Canonical discrimination (independent procedure) scatter plot of male *Lu. shannoni* measurements (all analyzed characters).

Figure 5. Canonical discrimination (independent procedure) scatter plot of female *Lu. shannoni* measurements (all analyzed characters).

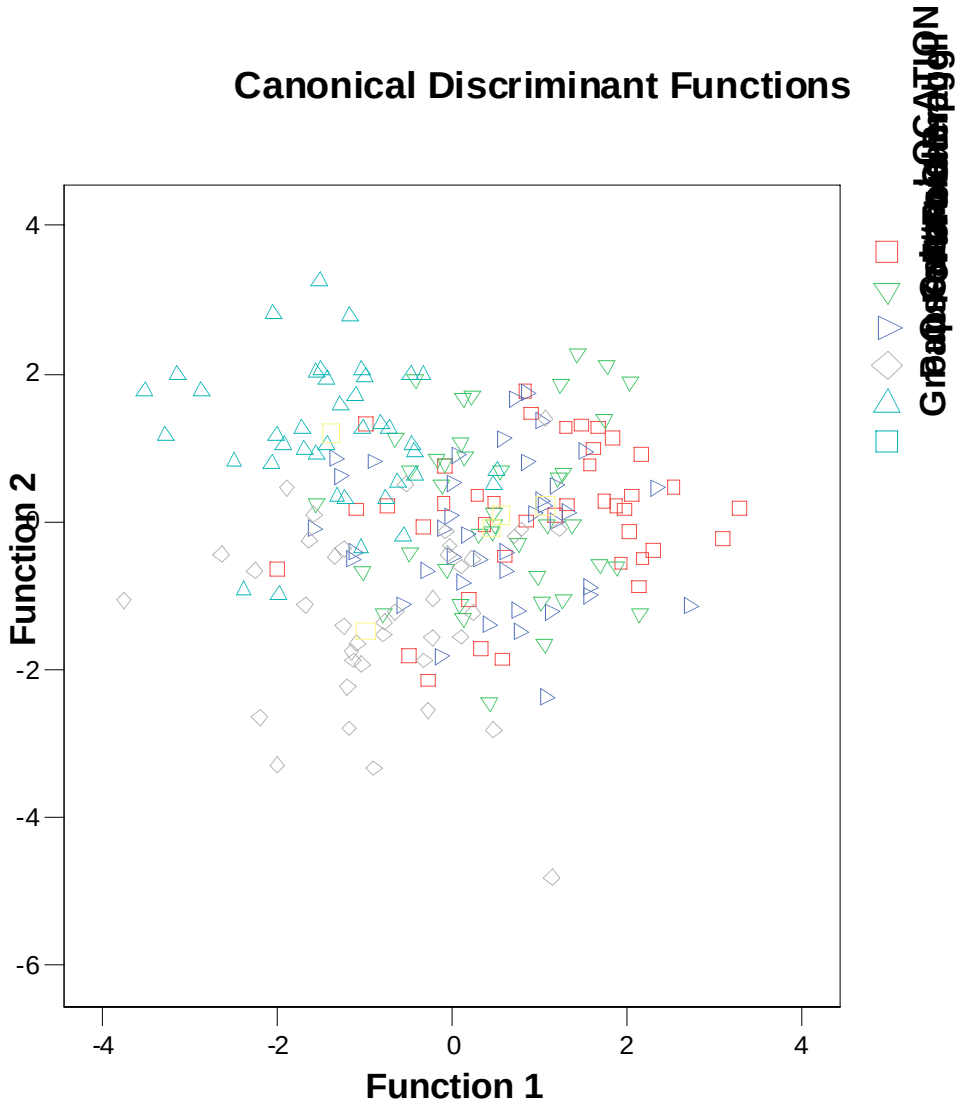

Figure 6. Canonical discriminant scatter plot of male characters not significant for test of homogeneity of variances (exclusion of palp2len, flag5len, and r5l) and the exclusion of all widths and ratios.

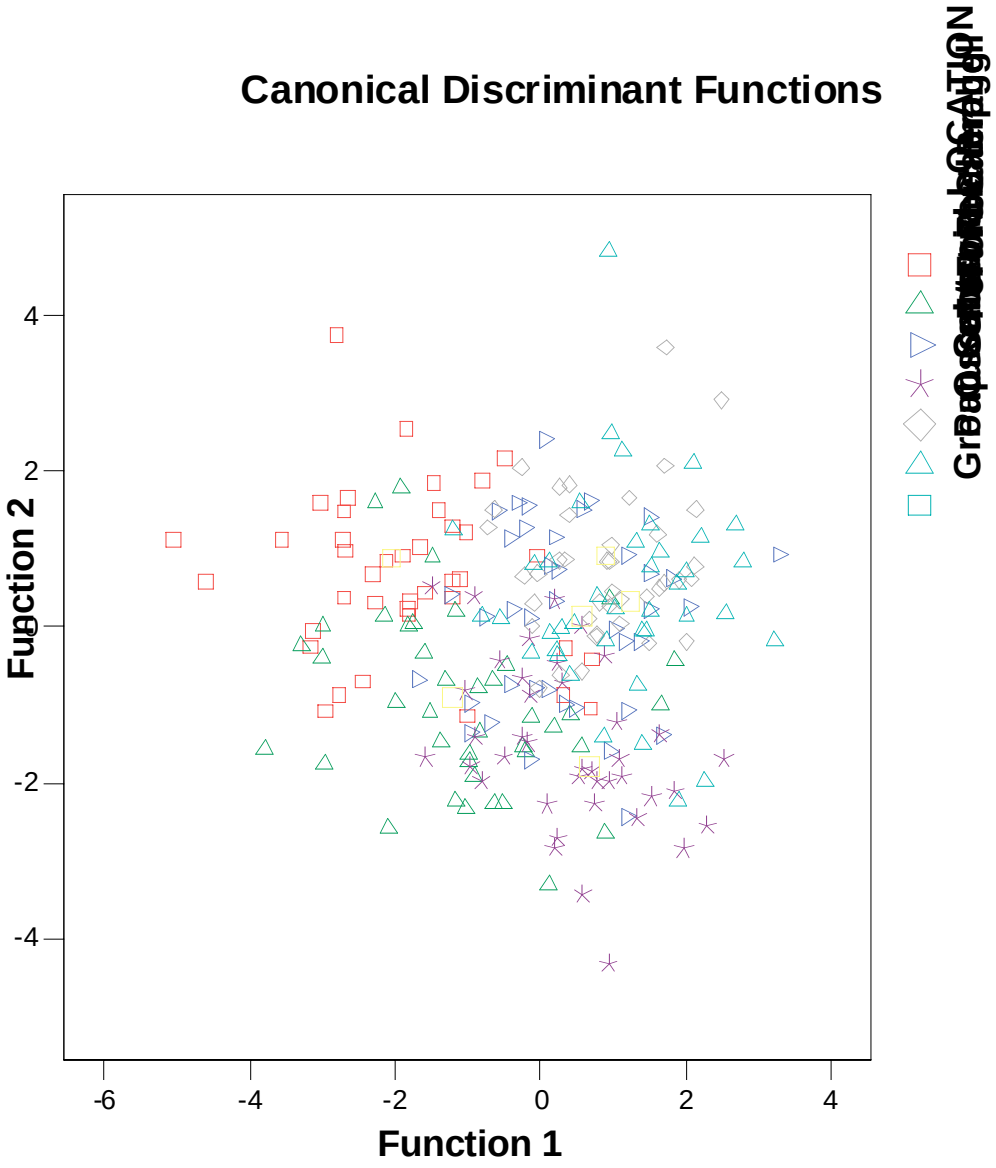

Figure 7. Canonical discriminant scatter plot of female characters not significant for test of homogeneity of variances (exclusion of hdlength, flag1len, r5l, alpha, and tibia) and the exclusion of all widths and ratios.

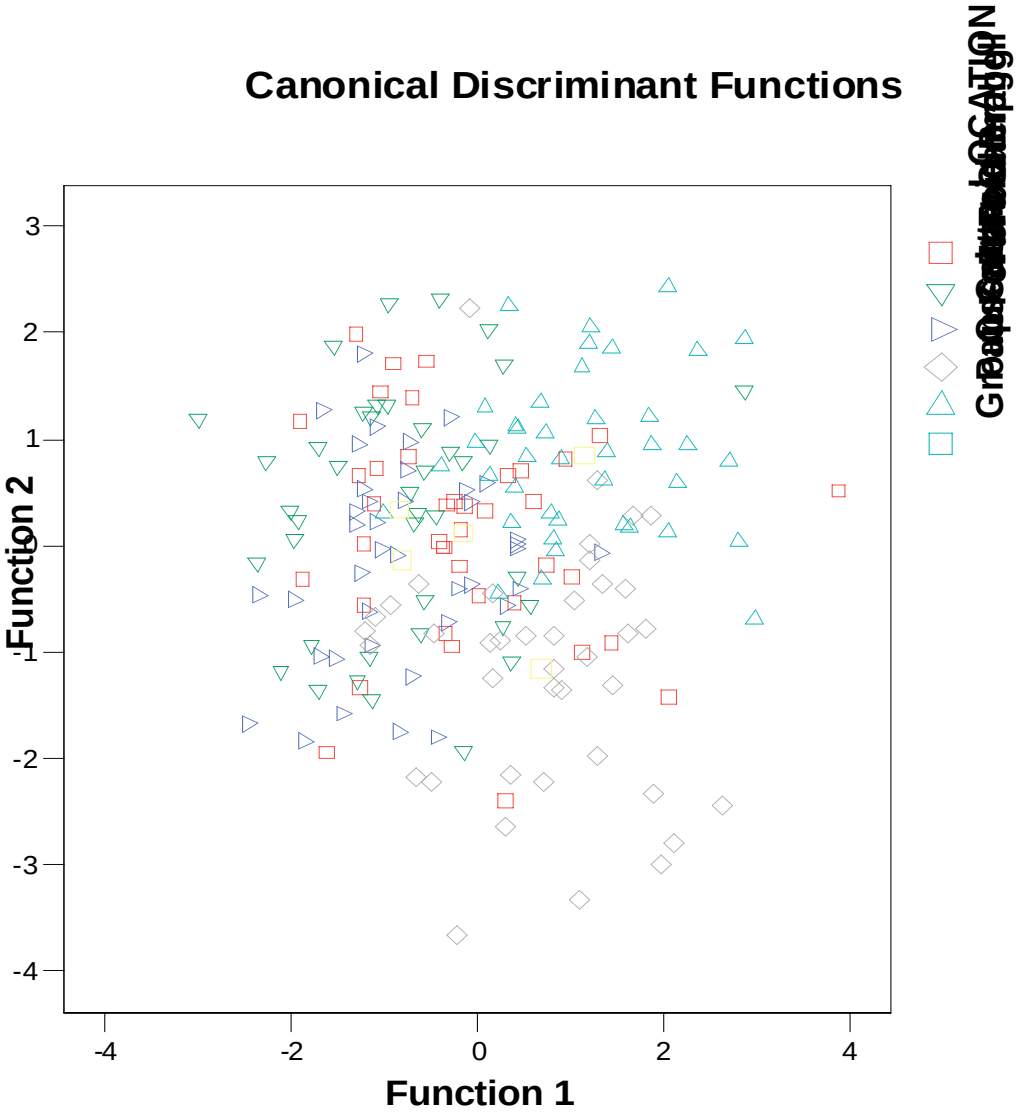

Figure 8. Canonical discriminant scatter plot of the male PCA scores of log-transformed characters (excluding widths and ratios) for all axes except the first.

Figure 9. Canonical discriminant scatter plot of the female PCA scores of log-transformed characters (excluding widths and ratios) for all axes except the first.

Chapter 3

Mitochondrial cytochrome c oxidase subunit 1 variation in *Lutzomyia shannoni* collected from seven geographical areas in the southeastern United States

ABSTRACT

Lutzomyia shannoni was collected at seven different geographical areas within the southeastern United States: Baton Rouge, LA; Fort Bragg, NC; Fort Campbell, KY; Fort Rucker, AL; Ossabaw Island, GA; Patuxent NWRR, MD; and Suwannee NWR, FL. The mitochondrial DNA marker cytochrome c oxidase subunit I (COI) was used to compare the purported populations. The 461-bp fragment of the COI gene amplified for 117 specimens of *Lu. shannoni* was without gaps and showed a polymorphism of 12.4%. From 57 variable sites, there were 23 parsimony-informative and 34 parsimony-uninformative. No insertions or deletions were identified within the aligned sequences. Within the total aligned sequence data set, an 87.6 % similarity resulted with 404 nucleotide positions constant. The intrapopulation variation matched the variation between the collection sites: percentage of identical residues among the sequences ranged from 100 to 97% for all pairwise comparisons in the total alignment and alignment of sequences from the respective collection site. An outgroup of 12 specimens did occur but the variation that separated the specimens from the main group was only on the order of 1% difference and all 12 sequences produced the same amino acids as the main grouping in a codon analysis. Results indicate that while there is variation, it is not sufficient to discriminate among the collection sites. The small amount of variation observed in the sequences did not have a diagnostic distribution and was not informative in distinguishing the specimens based upon collection site. This COI analysis indicates a single population of *Lu. shannoni* throughout the southeastern U. S. despite natural and human-made barriers to gene flow in this species of low vagility.

INTRODUCTION

Mitochondria are known as energy suppliers for the cell, as it is in the mitochondria that the transfer of electrons down an electrochemical gradient to the final acceptor of oxygen occurs with the subsequent production of energy. The cytochromes, large oligiomeric proteins, are embedded in the inner lipid bilayer membrane of the mitochondrion and act as carriers of the electrons (Karp, 1979; Keeton and Gould, 1986; Campbell et al., 1999). Cytochrome c oxidase subunit 1 (COI) is a polypeptide that serves as the terminal enzyme catalyst in the electron transport chain to the molecule oxygen thereby ensuring that a favorable chemiosmotic gradient will be maintained (Saraste, 1990). The COI in insects, as in nearly all animal phyla, is coded for by one of 37 genes contained in the circular, compact mitochondrial DNA (Hoy, 2003).

Mitochondrial DNA is the most commonly sequenced region in studies involving insect systematics and the COI is one of the most frequently sequenced segments within the mitochondrial DNA (Caterino et al., 2003). There are many reasons that drive the present popularity of mitochondrial DNA as a tool for studying genetic diversity, population structure, phylogeography, and population evolution: the relative ease at isolation/amplification and the subsequent straightforward analysis (Caterino et al., 2003), effective haploidy (Zhang and Hewitt, 1996), or the high copy number with thousands of mitochondria per cell, small genome size, maternal inheritance, lack of recombination yet fast evolution rate (Hoy, 2003). Avise (1994) stated the following: “Because of the maternal, nonrecombining mode of mtDNA inheritance and rapid

evolution in mtDNA sequence, the molecule often provides multiple alleles or haplotypes that can be ordered phylogenetically within a species, yielding intraspecific phylogenies (gene genealogies) interpretable as a matriarchal component of the organismal pedigree." When compared to other cytochrome subunits, the COI has a relatively large size and can therefore present the researcher with both highly conserved and variable regions making it an especially valuable tool in evolutionary studies (Morlais and Severson, 2002.)

While the advantages of using mitochondrial DNA are many, there are certain disadvantages to using mitochondrial genes. Lin and Danforth (2004) provided three cautionary notes when performing an evolutionary analysis based solely on mitochondrial genes: 1) an independent estimate of phylogeny is handicapped by the fact that all mitochondrial genes are linked on the same chromosome [presumably this creates essentially a single heritable unit that according to Hoy (2003) "produces gene diversity estimates that have larger standard errors than those determined using nuclear loci that can recombine" although future discoveries may indicate that in fact insects do exhibit recombination of mitochondrial DNA as has recently been revealed for scorpions (Gantenbein et al., 2005)] , 2) higher rate of substitution may not be able to resolve divergences older than 5 million years, and 3) mitochondrial genes tend to have high levels of homoplasy or convergent evolution, with the example of the extreme A/T bias across the insect taxa provided. Hoy (2003) cited possible biparental inheritance and introgression of mitochondria between species as complicating factors in the use of mitochondrial DNA. Lunt et al. (1996) found that different regions of the COI in the meadow grasshopper (*Chorthippus parallelus*) evolved at different rates and therefore sequence comparison in phylogenetic studies needed to be uniform. Zhang et al. (1996)

reported the presence of conserved mitochondrial sequences in the nuclear genome of the desert locust (*Schistocera gregaria*) with the warning that such a find in an experimental model could be a source of error when used as a molecular marker.

Brazin et al. (2006) conducted a meta-analysis to investigate if molecular markers conform to the principle of within-species diversity being dependent upon population size. The concept that animals with large population sizes such as invertebrates have a greater genetic diversity than smaller populations such as vertebrates is a central tenet of population genetic theory as it is predicted that neutral alleles will accumulate proportional to population size. Neutral alleles arise by a mutation that neither exerts an advantageous nor deleterious effect on the population but instead becomes established by chance and continue to exist by chance (Solbrig and Solbrig, 1979). The great amount of genetic variation observed between natural populations of organisms is generally attributed to neutral variation (Kimura and Ohta, 1971) and the measured variation in mitochondrial markers such as the COI is assumed to follow suit. The molecular markers that Bazin et al. (2006) examined were from three within-species polymorphism data sets: allozyme, nuclear sequences, and mitochondrial sequences. The authors found that nuclear and allozyme data were reflective of higher diversity in animals of greater abundance as predicted by population genetics but that mitochondrial DNA data were homogenous between major groups of taxa such as invertebrates and vertebrates. The conclusion reached is that natural selection must be driving a positive selection in the mitochondrial genome and ultimately decreasing the variability. The authors end by stating that mitochondrial DNA diversity is unpredictable and could be based on the last selective change rather than neutral differences displayed by demographic populations.

Given this, studies using mitochondrial DNA may be of limited value in assessing genetic and evolutionary relationships. Other studies on the question of neutrality in mitochondrial DNA have also indicated the interplay of natural selection (Gillespie, 1986; Ballard and Whitlock, 2004; de Stordeur, 1997).

Ballard and Whitlock (2004) argued that studies using only mitochondrial DNA as a marker in phylogeography research are fundamentally flawed. The authors pointed out that mitochondrial DNA may be prone to randomization by partial biparental inheritance, fixation of one haplotype due to fitness advantage (selective sweep), somatic mutations or nuclear pseudogenes of mitochondrial origin, and the possibility of introgression. The solution proposed is to "replicate across molecules", meaning to use other genetic markers such as in the nuclear genome to reach a concordance of results. Also recommended are tests of selection to ensure the mitochondrial marker conforms to the neutral equilibrium model.

Avise (1994) acknowledged the paucity of polymorphisms in mitochondrial DNA relative to neutrality expectations: "Most observed values fall orders of magnitude below theoretical expectations based on neutrality theory and present-day census population sizes (Nf values). In other words, despite extensive genetic heterogeneity, mtDNA diversity typically is much lower than neutrality theory predicts. Either mtDNA evolution is slower than generally believed or evolutionary effective population sizes are vastly lower than are present-day population sizes for most species." The author framed the dilemma as being part of the selection – neutrality debate and the difficulty of defining natural selection in molecular markers, yet continued to extol the advantages of

mitochondrial DNA in defining geographically localized clones and clades within many species.

Studies now incorporating both nuclear and mitochondrial genes provide complementary data for resolving phylogenetics as the two types of data are unlinked, having evolved under different constraints. Kiyoshi and Sota (2006) used the COI and several nuclear ribosomal RNA gene regions including the ITS2 sequences in the differentiation of Anisopteran dragonflies. Zhang et al. (2005) drew phylogenetic inferences from analyses of the COI and nuclear genes of two closely related Carabid beetles and concluded that the two types of markers can each provide different yet complementary information. In a study designed to end the reliance on phylogenetic data obtained solely from the mitochondrial genome, Arias and Sheppard (2005) included nuclear regions in resolving honey bee phylogeny. Larkin et al. (2006) used the same tactic with Andrenidae, another family within the Hymenoptera. Patsoula et al. (2006) combined morphological evidence with analyses from the COI and ITS2 to differentiate and characterize mosquito species in the *Aedes* genus. Dusfour et al. (submitted for publication Jan., 2006) in the study of *Anopheles sundaicus s. l.* compared three genetic markers: ITS2, cytochrome b, and the COI. While the ITS2 sequences were found unsuitable for discerning phylogenetic relationships, it did indicate introgression that was not revealed by the mitochondrial DNA. The use of the COI and the ITS2 in this study, as in the above cited studies, is a mitochondrial/nuclear complementary approach designed to provide greater resolving power compared to relying on any single marker.

The COI has been used extensively as a genetic marker in systematics research on mosquitoes. Dusfour et al. (2004) revealed the presence of cryptic species within

Anopheles sundaicus s. l. based on variability of both the COI and cytochrome b markers. This complex has a wide geographic distribution along coastal Southeast Asia and inhabits numerous islands that potentially represent isolated populations. The intrapopulational COI sequence diversities of populations from Vietnam, Thailand, and Malaysian Borneo ranged from 0.3% to 1.1% yet the interpopulation variation showed a polymorphism of 5.1%. The authors were able to conclude that two cryptic species inhabited the study sites based on gene sequences displaying genetic homogeneity within each collection entity yet differentiation between and unlikely gene flow between the sites. The maximum parsimony and neighbor joining analyses supported two separated clades when a tree was constructed of the evolutionary relationships of the specimens collected from the different geographical areas. Cook et al. (2005) differentiated *Aedes furcifer* and *Aedes taylori*, two morphologically identical cryptic species inhabiting West Africa, based on the variation of COI and COII sequences. The results indicated a low intraspecific variation and a high interspecific variation for both markers confirming their usefulness in tools to examine phylogenetic relationships at lower taxonomic levels.

Compared to mosquitoes, molecular mitochondrial data on sand flies is quite sparse. The cytochrome b mitochondrial DNA marker has been used in several publications pertaining to sand flies (Pesson et al., 2004; Torgerson et al., 2003; Parvizi et al., 2003; Testa et al., 2002; Esseghir et al., 1997) but there appears to be only two published papers centered on the COI. Arrivigalla et al. (2002) used the COI to compare twelve populations of *Lu. longipalpis* from Central and South America. The haplotypes produced were approximately 540 base pair fragments of the COIs that contained enough nucleotide diversity to separate out into four clades with three clearly representing

phylogenetic species. The clades identified were correlated with the geographic area of collection. Arrivillaga et al. (2003) expanded the study by increasing the number of examined populations to include the entire range of the species and by supplementing the COI analysis with the inclusion of the 12S and 16S mitochondrial DNA ribosomal sequences and the independent marker of isozyme loci. The results were in concordance with the previous paper as four clades with distinct geographic ranges were produced. When compared to the mitochondrial DNA ribosomal sequences, the COI revealed far greater levels of divergence which the authors attributed to the slower rate of evolution in the ribosomal genome of the mitochondria. The isozyme analysis was largely in concordance with the four distinct clades revealed by the COI marker.

MATERIALS AND METHODS

Information on the collection, identification, and preservation of specimens is detailed in the "Materials and Methods" section of Chapter 2.

Sample specimens:

Nucleic acids were extracted from individual sand flies by processing the remaining parts of the specimens used in the morphometric study of Chapter 2. As described in that chapter, each sand fly specimen used in the morphometric study was dissected with the head, one wing, one hind leg, and the terminal six segments of the abdomen mounted on a specimen slide. The remainder of the sand fly was assigned the same sample number as the slide-mounted features and placed in a cryovial containing 100% ethanol and stored at -70º C. Only the preserved samples of sand flies used in the morphometric study were processed for DNA extraction. A sample size of 20 specimens (10 males and 10 females) from each collection site (with the exception of Baton Rouge as only 13 *Lu. shannoni* sand fly specimens were collected at this site) was selected; the total number of specimens processed for the COI analysis was therefore 133.

DNA extraction, amplification, cloning, and sequencing:

Genomic DNA was purified using QIAGEN® DNeasy® Kit under protocol of QIAGEN® (2004). Each individual sand fly was homogenized in 180-ul of GIBCO™ Dulbecco's Phosphate-Buffered Saline (1X, .1 microcentrifuged) with an electric homogenizer and purified by the reagents included in the kit. An elution of 100-ul for

each individual sand fly was produced and stored at -20º C until amplification. The additional purification procedure of ethanol precipitation was conducted in preliminary trials but did not significantly improve results and was therefore not used in processing the sample specimens.

DNA amplification, cloning, and sequencing:

For all samples, amplifications were accomplished by using Invitrogen™ AccuPrime™ *Taq* DNA Polymerase System. All thermal cycling reactions were performed in a DNA Engine DYAD® Peltier Themal Cycler. PCR was carried out in a 25-ul total volume containing 20.75-ul distilled water (0.1 um filtered), 0.5-ul of Accuprime™ *Taq* DNA Polymerase, 1-ul of template, 0.25-ul of forward and reverse primer mix (10-uM each), and 2.5-ul of 10X AccuPrime™ PCR Buffer II. The protocol for thermal cycling of the COI fragment consisted of denaturing at 94º C (1 minute), annealing at 51º C (1minute) and extension at 68º C (1 minute). This cycle was repeated thirty five more times. A final extension at 68º C (2 minutes) occurred.

A fragment of the CO1 was initially amplified by using primers specified in Kambhampati and Smith (1995): (forward) 5'- TGA TCA AAT TTA TAA T - 3' and (reverse) 5'- GGT AAA ATT AAA ATA TAA ACT TC - 3'. When used by the above authors, the primers amplified an approximate 590 base pair (bp) fragment of the COI gene from a diverse range of insects including the families Drosophilidae and Muscidae of the Diptera order. In this study, samples of amplification product were initially run on 1.2% agarose gels with Tris Borate EDTA buffer at 70 volts (10 volts per centimeter of gel length) to confirm the presence of an ~ 520 bp product.

Confirmation that the primers amplified the target product effectively was achieved through the Invitrogen™ TOPO TA Cloning® ("one-step cloning strategy"): direct ligation of *Taq* polymerase-amplified PCR product into a plasmid vector (pCR®2.1-TOPO®), One Shot® chemical transformation into chemically competent *E. coli* (TOP10) cells, and then plating and recovery. All procedures were conducted according to Invitrogen™ (2004). Positive clones were incubated overnight on a shaker at 37º C in 5-ml of Luria-Bertani (LB) broth containing 1% ampicillin. A 4-ml volume from the LB sample was centrifuged at 3,000g for 1minute and the supernatant decanted. The pellet was then purified by using the QIAGEN® QIAprep® Spin Miniprep Kit as per protocol in QIAGEN® (2003). The plasmid was analyzed by use of EcoRI restriction enzyme that cut the insert from the vector; the separated vector and plasmid were then run on a 1.2% agarose gel to confirm the successful insertion by the presence of the expected band size of ~ 520 bp. A volume of 1-ul of uncut plasmid/vector was analyzed for DNA quantitaton by the Molecular Probes® Quant-iT™ DNA Assay Kit, High Sensitivity (Q33120). The assays determined the optimal volume of product from the miniprep procedure to include in the sequencing reaction mixture. The cloned inserts were then sequenced in the reverse direction with the M13 (Invitrogen) reverse primer in a dye terminator cycle using ABI Big Dye chemistry (Version 3.1) in an ABI 3100 Genetic Analyzer.

The produced fragment was compared to GenBank-deposited COI sequences via Basic Local Alignment Search Tool (BLAST) of GenBank (http://130.14.29.110/BLAST/), with the closest match being identified as the COI of *Drosophila jambulina* (AY757284.l). Currently there is no sand fly COI sequence

available in GenBank even though haplotypes have been published in peer-reviewed journals articles such as Arrivillaga et al. (2003). The amplified fragment was also compared to the haplotypes listed in Arrivillaga et al. (2003) for *Lu. longipalpis* and found to have large conserved segments between the compared sequences. Therefore, given the close match with the COI gene of *Drosophila* when "blasted" with GenBank-deposited sequences and the similarity of the published COI fragments of *Lu. longipalpis,* the amplified fragment was considered to be part of the target sequence of the COI gene.

While the Kambhampati and Smith (1995) primers did produce the target amplification, it was felt that the short lengths and low GC concentrations could lead to specificity problems in latter amplifications. The primers were used as a basis for the construction of designed primers that more closely conformed to the guidance of primer design as stated in McPherson and Moller (2000). The designed primers constructed were the following: (forward) 5'- TTA TAA TGT AAT TGT TAC AGC C - 3' and (reverse) 5'- AAA TAT AAA CTT CTG GAT GTC C - 3'. Essentially, the Kambhampati and Smith (1995) primers were modified by extending the length based on the cloned sequences and then trimming from the ends until an appropriate primer resulted. Confirmation that the designed primers amplified product effectively was achieved through the Invitrogen™ TOPO TA Cloning® ("one-step cloning strategy") as previously described. All products for the COI analysis were amplified from the above designed primers.

PCR products were refined by the use of Edge Bio Systems™ PERFORMA® Spin Columns that consisted of a gel matrix designed to remove salts, amino acids, nucleotides, traces of solvents, and other low molecular weight materials from the

product when placed under the low speed of a microcentrifuge. Gel purification of the amplified product by excising the target DNA fragment from an agarose gel and then purification via QIAGEN® QIAquick® Gel Extraction Kit did not significantly improve preliminary trials and was therefore not used.

Direct sequencing of the PCR products to a dye terminator cycle was initiated to conserve financial resources and time as the number of processed specimens (133) was large. Successful direct sequencing of the amplified product was achieved as the direct sequences were found to be of the same quality as the cloned sequences, and therefore the cloning, miniprep, and DNA quantitation steps could be omitted. Direct sequencing of the samples was conducted by having each product split into two 10-ul volumes with both undergoing a dye terminator cycle sequencing reaction when added to 1-ul of either a forward or reverse internal primer (10-pmol), 4-ul of Terminator Ready Reaction Mix, and 5-ul volume of deionized water to make a 20-ul total volume. The internal primers were the following: (forward) 5'- TGG ATG TCC AAA AAA TCA AAA TAG GTG - 3' and (reverse) 5'- TGT TAC AGC CCA TGC TTT TGT AAT - 3'. After completion of the cycle sequencing, each individual product was again refined with an Edge Biosystems™ PERFORMA Spin Column. Direct sequencing was completed by generating a forward and reverse DNA strand from each sample using ABI Big Dye chemistry (Version 3.1) generated with an ABI 3100 Genetic Analyzer.

Data analysis:

Forward and reverse sequences of each sand fly mitochondrial COI DNA were aligned and combined by the default settings in Contig Express, a component of Invitrogen™ Vector NTI Advance 10.0.1 (Static License 2005 Invitrogen™

Corporation), and manually adjusted for obvious errors and misalignments. The consensus strand produced from each individual sand fly was then aligned with all other produced consensus strands by the default settings in Invitrogen™ Vector NTI Explorer. The alignment was created using the Clustal W algorithm that basically calculated a crude similarity between all pairs of sequences and then constructed a dendrogram that provided the multiple alignment stage the order in which to align the sequences for the final multiple alignment. The sequences were then aligned in larger and larger groups until all sequences were incorporated in the final alignment.

A codon analysis of the COI sequences was conducted with Invitrogen™ NTI Explorer since the COI gene in the mitochondrial DNA of the cell is actively transcribed into mRNA and subsequently translated into amino acids. This analysis determines if nucleotide substitutions observed among the aligned sequences result in an amino acid change (and ultimately a conformational change in the resulting protein) or are "silent" with a different codon still coding for the same amino acid and ultimately the same protein.

The nucleotide sequences were exported to the CLUSTAL W software (http://bioweb.pasteur.fr/seqanal/interfaces/clustalw-simple.html),version 1.83, (Thompson et al., 1994) for formatting/data entry into the PHYLIP package, Version 3.6 (Felsenstein, 2004) and into PAUP version 4.0b10 (Swofford, 2002). The PHYLIP package produced 1,000 data sets derived by bootstrap resampling using the SEQBOOT program. These data sets were then entered in the DNAPARS program with 10,000 unrooted trees produced based on the parsimony method of phylogeny estimation. The file containing all of the possible trees as derived by DNAPARS was then entered into

the CONSENSE program which computed a consensus tree by the majority–rule consensus tree method. The consensus tree was prepared as a figure with DRAWTREE application and color coded to distinguish collection sites by the Adobe® Illustrator® 9.0.1 program. Genetic distances, quantitative estimates of genetic divergence, were determined both between and within collection sites by the distance matrix program Kimura's 2-parameter model in the DNADIST program.

RESULTS

The 461-bp fragment of the COI gene amplified for 117 specimens of *Lu. shannoni* was without gaps and showed a polymorphism of 12.4% (Appendix E). From 57 variable sites, there were 23 parsimony-informative and 34 parsimony-uninformative. No insertions or deletions were identified within the aligned sequences. Within the total aligned sequence data set, an 87.6 % similarity resulted with 404 nucleotide positions constant. A total of 133 sequences were initially obtained but 16 were deleted from the analysis due to significantly shorter lengths because of "noise" in the amplification procedure. The relationship among the sequences is represented in Figure 1 produced by using the sequence distance method Neighbor Joining (NJ) algorithm of Saitou and Nei (1987) in the Invitrogen™ Explorer NTI program. An identity table (not shown) displaying the percentage of identical residues among the sequences ranged from 100 to 97% for all pairwise comparisons in the total alignment. When sequences were grouped by collection site and then separately aligned within their respective group, the collection sites of Patuxent NWRR, Suwanne NWR, and Fort Bragg displayed 100 to 97%; the Fort Campbell site displayed 100 to 98%; while Fort Rucker and Ossabaw Island displayed 100 to 99% of identical residues among the sequences for all pairwise comparisons. The distance matrix program Kimura's 2-parameter genetic distance model was in concordance with the identity table as interpopulational (between collection sites) and intrapopulational (within collection sites) diversities did not exceed 3%.

While the Invitrogen™ NTI Explorer program provided a sense of the topology of the relationship between the sequences, a statistical estimate of the reliability of the groupings was made by bootstrapping (Hall, 2001) 1,000 replicates using the PHYLIP and PAUP packages. Figure 2 provides the consensus tree derived from the PHYLIP package, Version 3.6, using SEQBOOT, DNAPARS, and CONSENSE programs and the DRAWTREE application. Figure 3 provides an enlarged view of the left portion of the tree from Figure 2 with color codes to distinguish the specimens from each of the various collection sites. Bootstrap values, as derived in PAUP from 1,000 bootstrap replications with 50% majority-rule consensus tree, ranged from 54 to 62% in the main portion of the tree and a 95% bootstrap value supported the outgrouping of the 12 specimens.

The reading frame based on the invertebrate mitochondrial genetic code in Invitrogen™ NTI Explorer was used to translate the COI sequences into amino acids. The translated protein sequences were 153 amino acids in length from the -3 translation frame of the complementary strand. This translation frame did not contain any stop codons indicating that the data was free of nuclear pseudogenes. In the alignment of all translated sequences, there were 6 positions that induced different amino acids with all others silent. The 6 positions were from 6 different sequences (specimens) from the following collection sites: 2 from Baton Rouge (BR005 at position 61, a threonine translated vice alanine; BR006 at position 100, a valine translated vice isoleucine), 1 from Patuxent (PX017 at position 109, an isoleucine translated vice valine), 1 from Suwannee NWR (SU014 at position 42, an alanine translated vice valine), 1 from Fort Rucker (FR004 at position 65, a threonine translated vice alanine), and 1 from Ossabaw Island (OS005 at position 114, a methionine translated vice valine).

The BLAST of GenBank (http://130.14.29.110/BLAST/) was used to find regions of local similarity between the consensus strand of the translated protein sequences and the protein sequences entered in the databases. This was conducted to determine the location of this study's COI segment in relation to what has previously been studied and entered into the GenBank databases. The first three "hits" (statistically significant matches) were from Dipteran species with proteins of approximate lengths of 500 amino acids long. The consensus protein sequence form this study aligned between the 74 and 229 positions. Within this region of alignment, the consensus positions between the 4 protein sequences were 92.2% identical; there were 12 amino acid differences (2 unique to to the consensus sequence).

An ANOVA comparing the morphological characters used in Chapter 2 was conducted in SPSS® 12.0.1 for Windows of the 12 specimens in their respective gender. The 6 male specimens that separated from the main group in the COI sequences were compared with all other male specimens: flagellomere VII width was found to be significantly wider at the 0.05 alpha level. For the females, the ANOVA results were significant for two characters: radial 5 vein length and papal segment I width. In both characters, the pooled 6 specimens displayed larger measurement values when compared to all of the other specimens.

DISCUSSION

Phylogenetic analyses of the COI sequenced region of *Lu. shannoni* from seven sites within the southeast U.S. does not support significant divergence among the subject populations. Intrapopulational sequence diversity ranged from 1 to 3% per site which matched the interpopulational sequence diversity. The codon analysis indicated that the vast majority of the nucleotide substitutions were silent as only six amino acid changes were evident. In essence, the low within-population haplotype variation was observed again when the haplotypes were compared across the collection sites. While several unique haplotypes could be discerned, there was no grouping of these haplotypes to any of the various collection sites. Unlike the results reported in Arrivillaga et al. (2003) for the COI of *Lu. longipalpis* populations, large nucleotide divergences ranging from 9 to 10% were not observed. A group of specimens does separate out in both Figures 1 and 2, although the nucleotide divergence between the group of 12 and the major group is only on the order of 1% difference. Figure 3 shows that the specimens from the various collection sites did not group together as would be expected if the sand flies from the collection sites were of separate populations.

It is interesting to examine the 12 sand fly specimens that separated from the main grouping as shown in Figure 2. This grouping was supported by a 95% bootstrap value with 1,000 replications run in the parsimony program of PAUP and therefore confidence

in the grouping is high. No specimen among this "outgroup of 12" displayed an amino acid change when compared to the main grouping in the codon analysis and therefore the all nucleotide changes were were "silent". The group shares six polymorphic sites: position numbers 7, 55, 67, 288, 403, and 442 as shown in Appendix E. The polymorphism at position number 403, an adenine substitution for guanine, is unique to the group. Again, being aware that the divergence is based only on a 1% nucleotide difference, what additional characters do these 12 specimens have in common that distinguish them from the main grouping? The 12 specimens are from three different collection sites: Fort Bragg (3 males and 2 females), Patuxent NWRR (3 males and 3 females), and Suwannee NWR (1 female). All three of these sites are eastern seaboard locations situated in the Outer Coastal Plain Mixed Province ecosystem (see Figure 1 of Chapter 2). While speculation can be made that perhaps there exists a greater gene flow between these sites as compared to the total gene flow between all sites, sampling sizes used in this study may simply not have been large enough to detect similar divergent COI sequences from the other collection sites. Collection times did vary (see Chapter 2) as the Fort Bragg specimens were collected in early September, the Suwannee NWR specimen was collected in late May, and the Patuxent NWRR specimens were collected in the time range of June – August, 2005. The issue of seasonal emergence and possibility of sampling different generational populations can be considered to be minor as the separated group of 12 was collected in the same time ranges as all other specimens in the major grouping. The ANOVA comparing the specimens with all other specimens of that respective gender did reveal that the separated group was significantly larger for a

total of three morphological characters but this finding is diminished when one considers the large number of characters found to be significant (see Chapter 2).

It was expected that the specimens from Ossabaw Island would show the greatest divergence in comparison to other locations given the low vagility of the species and the isolated, island ecosystem. This expectation did not come to realization as the Ossabaw Island specimens were distributed throughout Figure 3 instead of grouping into a branch by themselves. Evidently, there is either adequate gene flow between the island and mainland sand flies or the time period for polymorphisms to accrue is of such a long duration that no significant divergence has occurred since the last time the island and the mainland were in close enough proximity for the sand flies to disperse back and forth.

A corollary to this issue is the fragmentation of the old growth hardwood habitat throughout the southeastern U.S. and the presumed decrease in the genetic variability of *Lu. shannoni* due to genetic drift. In the allozyme study of Mukhopadhyay et al. (2001) that examined *Lu. shannoni* in Colombia, no significant genetic variability was detected between collection sites delineated by discontinuous forest habitat. However, the authors did find genetically distinct populations where the Andean mountain chains separated collection sites. In this study, every one of the collection sites can be considered to be part of a fragmented, patchy, discontinuous forest habitat that could in theory isolate the sand flies on “islands” of habitat in a sea of human-modified landscapes. Yet, no significant divergence of the COI was observed between any of the collection sites. Even natural barriers such as the open water surrounding Ossabaw Island and the Appalachian mountain chain separating Fort Campbell from the other sites, did not result in significant COI divergence.

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Figure 1. Relationship among the CO1 sequences as represented by the sequence distance method Neighbor Joining (NJ) algorithm of Saitou and Nei (1987) as calculated in the Invitrogen™ Explorer NTI program. Scores of the crude similarity between all pairs of sequences ("Parities alignment") are shown in parentheses following each specimen number. BG: Fort Bragg, BR: Baton Rouge, FC: Fort Campbell, FR: Fort Rucker, OS: Ossabaw Island, SU: Suwannee NWR, PX: Patuxent NWRR

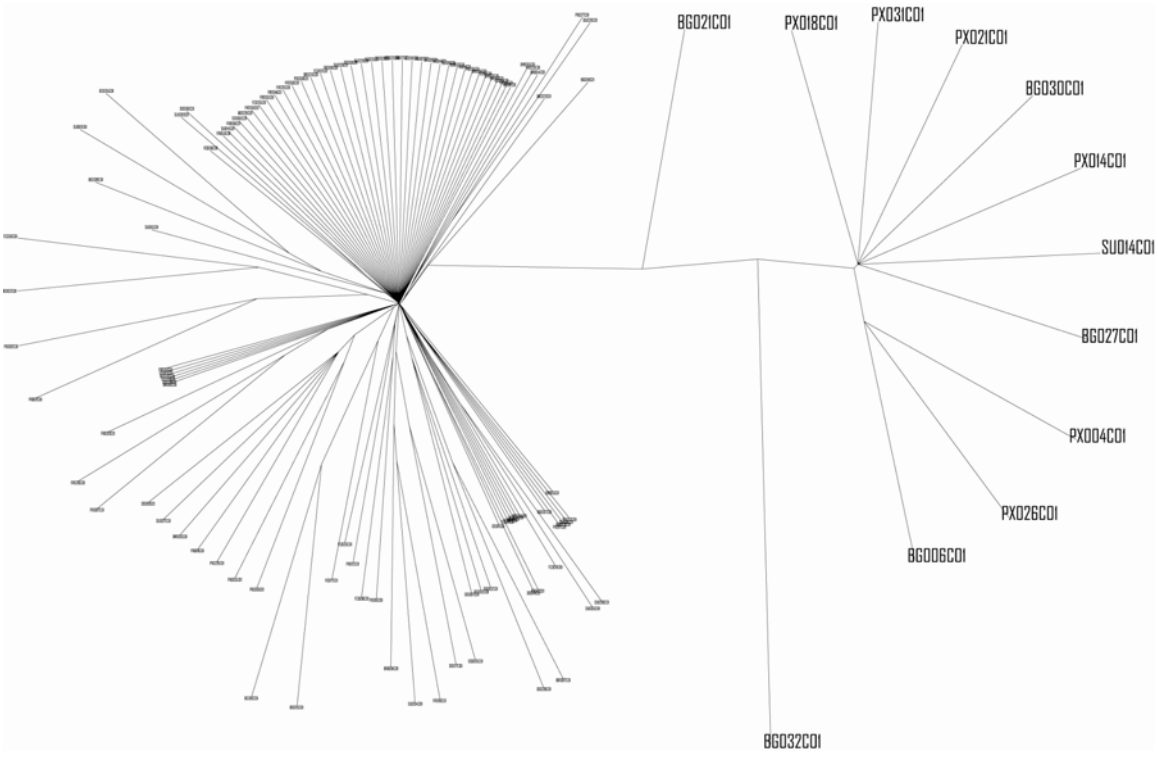

Figure 2. Unrooted parsimony tree based on *Lu. shannoni* CO1 sequences as produced from the DNAPARS program (bootstrap analysis with 1,000 replicates) of the PHYLIP package.

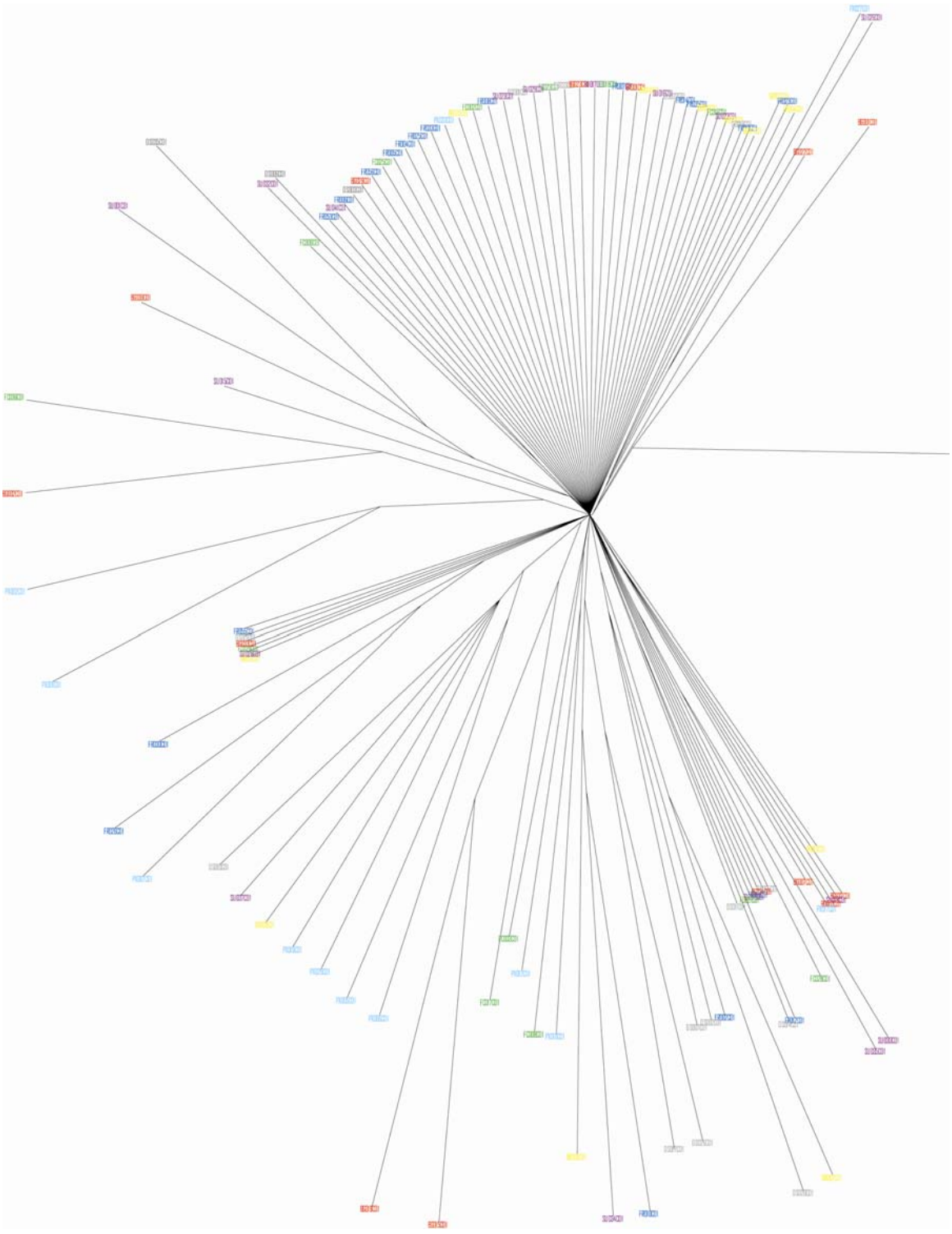

Figure 3. Enlarged section of COI sequence tree from Figure 2 (left group) highlighted with color codes to distinguish collection sites.
Patuxent NWRR: turquoise
Ossabaw Island: gray
Fort Rucker: blue
Suwannee NWR: violet
Fort Campbell: green
Fort Bragg: red
Baton Rouge: yellow

Chapter 4

Internal Transcribed Spacer 2 variation in *Lutzomyia shannoni* from seven geographical areas in the southeastern United States

ABSTRACT

Intragenomic heterogeneity of the Second Internal Transcribed Spacer (ITS2) was investigated in the sand fly *Lu. shannoni* from seven geographic locations in the southeastern United States to assess the possible existence of a cryptic species complex. The collection sites were: Baton Rouge, LA; Fort Bragg, NC; Fort Campbell, KY; Fort Rucker, AL; Ossabaw Island, GA; Patuxent NWRR, MD; and Suwannee NWR, FL. Polymerase chain reaction-amplified copies of a 229-bp section of the ITS2 was sequenced among 42 specimens (6 specimens from each of the seven collection sites) and then entered into phylogenetic analysis with the single GenBank sequence for the species. The sequence divergence (Kimura's 2-parameter genetic distance) within collection sites ranged from 0 to 1.8% while the inter collection site genetic distances ranged from 0 to 2.7%. The small amount of variation observed in the sequences did not have a diagnostic distribution and were not informative in distinguishing the specimens based upon collection site. Results indicate that at the locations examined there exists no significant ITS2 variation. A single population of *Lu. shannoni* may exist throughout the southeastern U.S. but other molecular markers and morphological data are needed to confirm this speculation.

INTRODUCTION

The Second Internal Transcribed Spacer (ITS2), located between the 5.8S and 28S ribosomal RNA (rRNA) coding regions on the eukaryotic ribosomal DNA (rDNA) repeat units, provides signals in the transcriptional processing of rRNA (Hillis and Dixon, 1991). As a noncoding segment, the ITS2 generally has a higher degree of polymorphism than the more conserved 18S, 5.8S, and 28S coding regions of the transcription unit (Hoy, 2003) and is therefore particularly useful in discerning the phylogeny among closely related taxa such as cryptic or sibling species. The ITS2 has high interspecific variability when compared between two similar species yet retains a low degree of intraspecific variability thereby allowing the grouping of taxa into a species and/or population. In addition the ITS2, along with the entire rDNA repeat units, lends itself to comparatively easy analysis by being in great abundance within a cell and thus an easy target for amplification and sequencing (Caterino et al., 2000). The actual ribosomal gene copy number per cell is quite variable in insects depending upon the species (Hoy, 2003), but the fact that a substantial number of multiple copies are present, usually ranging into the thousands of copies in every cell of the organism, gives the researcher a most valuable tool in the ITS2.

Literature from the molecular biology arena is replete with publications pertaining to ITS2 sequences and the topics of phylogeny, evolution, and population diversity. In the published realm of insect research, the internal transcribed spacers and the 28S regions predominate as PCR targets for discerning relationships between species within the Diptera and Hymenoptera orders (Caterino et al., 2000). Over the last twenty years there

has been extensive use of rDNA for differentiation of the *Anopheles* genus (Collins and Paskewitz, 1996) with applications now appearing for other mosquito genera (Vinogradova and Shaikvich, 2005; Behbahani et al., 2005). Wilkerson et al. (2004) used not only sequence variability, but also sequence lengths of the ITS2 to discriminate between 6 sympatric species in the mosquito *Anopheles crucians* (*sensu lato*) complex from populations in the southeastern United States. Comparisons of amplified ITS2 sequences revealed that a taxon typed *Anopheles anthrophagus* inhabiting China was actually in synonymy with *Anopheles lesteri*, a taxon inhabiting South Korea and the Philippines and therefore the separation on morphological grounds was flawed (Wilkerson et al., 2003). Hackett et al. (2000) amplified the ITS2 sequences of two sibling species of Afrotropical *Anopheles*: *Anopheles rivulorum* and *Anopheles funestus*; the results indicated the morphologically similar species were in fact highly diverged as the ITS2 sequences could not even be aligned except for a 26 base pair fragment between the 520 base pair sequence of *Anopheles funestus* and the 840 base pair sequence of *Anopheles funestus*. Seeking an alternative to the complex and problematic RAPD-PCR technique, Li and Wilkerson (2005) devised a type of dichotomous key based on sequence product length to differentiate the Neotropical *Anopheles (Nyssorhynchus) albitarsis* by amplifying the ITS2 and sorting to species primarily on the basis of the amplified product length.

While not as dominant as seen in the literature pertaining to mosquito phylogeny, the ITS2 has distinguished spatial clusters of biogeographical populations and revised phylogenies within *Phlebotomus.* (Interestingly, very little work with the ITS2 appears to have been conducted on New World sand flies as the literature search engines come up

empty when prompted with such topic searches. Many authors in the sand fly literature lament the miniscule amount of molecular studies on sand flies in general when compared to mosquitoes; this may be due in large part to finite financial resources being allocated to what is perceived as the more pernicious vector, than sand fly researchers refusing to accept contemporary techniques. Torgerson et al. (2003) stated the point that although many *Phlebotomus* species have been studied with molecular data, for *Lutzomyia*, only a few species have been examined and "since molecular data is still limited for New World species, the genetic relationships among accepted morphologic groups with the *Lutzomyia* genus are poorly known.") Variation in the ITS2 has augmented morphological studies in the clarification of *Phlebotomus* phylogenies of species inhabiting Madagascar (Depaquit et al., 2002a; Depaquit et al. 2004) and the Mediterranean region (Di Muccio et al., 2000). Depaquit et al. (2002b) used small sample sizes of up to three *Phlebotomus* specimens collected from various locations throughout Europe and Northern Africa, but was still able to discriminate, via amplification of the ITS2, four closely related species and two sister-group populations of one species. *Phlebotomus sergenti*, a vector species of *Leishmania tropica*, was shown on the basis of bootstrap values to have probable divisions into sister populations within the geographic range. The regions that these sister populations inhabited also correlated to areas of differences in the transmission of *Leishmania tropica*, although the authors were quite careful to limit their speculation and state that other factors than genetic differences could be responsible. Depaquit et al. (2000), in perhaps the first full study to use the ITS2 to address sand fly phylogeny, found that the ITS2 could successfully differentiate several species within the subgenus *Paraphlebotomus*.

Other areas of the ribosomal DNA unit such as the 18S and 5.8S ribosomal DNA sequences have also been used to infer phylogenetic relationships within the infraorder Culicomorpha of Diptera (Miller et al., 1997), but there appears to be limited utilization of these units in sand fly phylogeny studies at the species level presumably due to the conserved nature of the segments. One study that did use the small subunit (16 – 18S rRNA) to compare relationships within the phlebotomine sand flies at the subgenus and species level could not decipher any intraspecific markers between geographical populations (Aransay et al., 2000), probably as a result of being slower evolving sequences than the ITS2. Use of the mitochondrial small subunit 12S rRNA provided questionable results in determining systematic relationships among *Lutzomyia*, and while the nuclear 28S fared better, this method also encountered problems on certain species relationships (Beati et al., 2004).

MATERIALS AND METHODS

Information on collection, identification, preservation, and processing of specimens is detailed in the "Materials and Methods" section of Chapter 2. In-depth information on the DNA extraction, cloning, and sequencing methods are detailed in the "Materials and Methods" section of Chapter 3.

DNA amplification, cloning, and sequencing:

The ITS2 was amplified by using the following designed primers: (forward) 5'- ACT GCA GGA CAC ATG AAC ATC - 3' and (reverse) 5' - CCT GGT TAG TTT CTT TTC CTC C - 3'. These primers were constructed by first aligning appropriate target sequences from other sand fly species (listed with GenBank accession numbers): *Phlebotomus tobbi* (AF205523), *P. ariasi* (AF205525), *P. andrejevi* (AF218315), *P. mongolensis* (AF218320), *P. saevus* (AF218322) *P. perniciousus* (AF205526), *P. longicuspis* (AF205526) and *P. perfiliewi perfiliewi* (AF205527). In addition, the complete sequences of the 5.8 S ribosomal RNA gene, ITS1, ITS2, and partial sequences of 18S and 28S rRNA genes of *Lu. shannoni* (U48382) as submitted in GenBank by Miller et al. (1997) was used to determine highly conserved regions flanking the target ITS2 among *Lu. shannoni* and the different sand fly species. Once these conserved flanking regions were identified, the above primers were designed from within the regions according to primer design guidance in McPherson and Moller (2000). Given that the conserved regions existed between species and even genera, the assumption was made that the primers would anneal to the template of all the *Lu. shannoni* samples as the species displayed the same conserved regions. A number of designed variations of the

forward and reverse primers were tried with the best results being achieved with the designed primers listed above.

ITS2 primers used by Depaquit et al. (2000) in molecular systematics research on *Phlebotomus* species were initially attempted to obtain product from *Lu. shannoni* but results were unsuccessful even when the product was cloned. The primers and amplification profile used by Porter and Collins (1991) that successfully amplified the ITS2 with flanking regions in *Anopheles* was also tried, but had limited success in this study.

Samples of amplification product were initially run on 1.2% agarose gels with Tris Borate EDTA buffer at 70 volts to confirm the approximate 250 bp product. Confirmation that the designed primers amplified product effectively was achieved through the Invitrogen™ TOPO TA Cloning® (one-step cloning strategy) as detailed in Chapter 3. Trial runs indicated that the amplified sequence was nearly identical to the published ITS2 sequence in GenBank and therefore confirmation that actual target sequence was amplified by the designed primers was achieved.

For all samples, amplifications were conducted by using Invitrogen™ AccuPrime™ *Taq* DNA Polymerase System. All thermal cycling reactions were performed in DNA Engine DYAD® Peltier Thermal Cycler. The protocol for thermal cycling consisted of denaturing at 94º C (1 minute), annealing at 62º C (1minute), and extension at 68º C (2 minutes). This cycle was repeated four more times. A denaturing at 94º C (1 minute), annealing at 58º C (1 minute), and extension at 68º C (2 minutes) then took place with the cycle repeated four more times. A denaturing at 94º C (1 minute), annealing at 54º C (1 minute), and extension at 68º C (2 minute) then took place and was repeated for twenty

four more times. A final extension at 68º C (10 minutes) occurred. This temperature and time profile produced the best results compared to a number of variations including a "touchdown PCR" where the annealing temperature was reduced by 1º C every two cycles, moving from 64 to 54º C over the first 20 cycles with annealing times being 30 seconds and extension temperatures at 72º C for 1 minute.

PCR products were refined by the use of Edge Bio Systems™ PERFORMA® Spin Columns consisting of a gel matrix designed to salts, amino acids, nucleotides, traces of solvents, and other low molecular weight materials from the product when placed under the low speed of a microcentrifuge. Gel purification of the amplified product by excising the target DNA fragment from a 1.2% agarose gel and then purification via QIAGEN® QIAquick® Gel Extraction Kit did not significantly improve preliminary trials and therefore direct sequencing of the products was selected as the method. Direct sequencing of the samples was conducted by having each product split into two 10-ul volumes with both undergoing a dye terminator cycle sequencing reaction when added to 1-ul of either a forward or reverse internal primer (10 pmol), 4-ul of Terminator Ready Reaction Mix, and 5-ul volume of deionized water to make a 20-ul total volume. The internal primers were the following: (forward) 5'- CAT CGA CAT TTT GAA CGC ATA TTG C- 3' and (reverse) 5'- GGT AGT CAC ATA TGA GTT G – 3'. After completion of the cycle sequencing, each individual product was again refined with an Edge Bio Systems™ PERFORMA® Spin Column. Direct sequencing of each sample was completed on an ABI Big Dye chemistry (Version 3.1) generated with and ABI 3100 Genetic Analyzer.

Data analysis:

The planned methodology entailed sequencing in both directions but early results indicated that the reverse internal primer was not functioning effectively as the produced reverse strands were of poor quality with regions of the product skewed with multiple nucleotide signals. A number of other reverse internal primers were tried but all resulted in the same "noisy" chromatograms. Different primers, temperature profiles, denaturation times, annealing times, and extension times were also experimented with, but all resulted in reverse strands that would not assemble with the forward strand due to the excessive competing signals contained within the reverse strand. Due to financial and time constraints, it was decided to perform the cycle sequencing twice with the forward internal primer and use the two forward strands for initial alignment and combination into a consensus strand representing the individual sand fly specimen. Sequences of the ITS2 were compared to the GenBank ITS2 segment of *Lu. shannoni* (U48382) and to a cloned ITS2 fragment from specimens of the Patuxent NWRR. The two forward sequences of each sand fly were aligned and combined by the default settings in Contig Express, a component of Invitrogen™ Vector NTI Advance 10.0.1 (Static License 2005 Invitrogen Corporation), and manually adjusted for obvious errors and misalignments. The consensus strand produced from each individual sand fly was then aligned with all other produced consensus strands by the default settings in Invitrogen™ Vector NTI Explorer.

A total of 42 *Lu. shannoni* (6 specimens from each of the seven collection sites) were entered into the analysis (process described in Chapter 3) in addition to GenBank sequence number U48382 as submitted by Miller et al. (1997). Certain sequences were

trimmed up to 5 bp on the 3' end in order to have a uniform length of 229-bp comparisons [the sequence length for the full ITS2 entered in GenBank by Miller et al. (1997) is 234-bp length]. After alignment, the sequences were exported to the CLUSTAL W software (http://bioweb.pasteur.fr/seqanal/interfaces/clustalw-simple.html), version 1.83, (Thompson et al., 1994) so that the sequences could be formatted for data entry into the PHYLIP package, Version 3.6 (Felsenstein, 2004) and into PAUP version 4.0b10 (Swofford, 2002). In the PHYLIP package, 1,000 data sets were derived by bootstrap resampling using the SEQBOOT program. These data sets were entered in the DNAPARS program where the input order was "jumbled" 100 times and 10,000 unrooted trees were produced based on the parsimony method of phylogeny estimation. The file containing all of the possible trees as derived by DNAPARS was then entered into the CONSENSE program which computed a consensus tree by the majority–rule consensus tree method. The consensus tree was prepared as a figure with DRAWTREE application and color-coded to distinguish collection sites by the Adobe® Illustrator® 9.0.1 program. Genetic distances, quantitative estimates of genetic divergence, were determined both between and within collection sites by the distance matrix program Kimura's 2-parameter model in the DNADIST program.

RESULTS

The 229-bp alignment of the ITS2 sequences derived from 42 specimens of *Lu. shannoni* is provided in Appendix F. A polymorphism of 3.5% with 8 variable sites (counting gaps as missing data), 4 parsimony-informative and 4 parsimony-uninformative, is displayed. Within the aligned sequence data set, a figure of 94.8% similarity resulted with 217 nucleotide positions constant accounting for gaps. Ten distinct haplotypes were resolved from the total aligned sequences. The relationship among the sequences is represented in Figure 1 by using the sequence distance method Neighbor Joining (NJ) algorithm of Saitou and Nei (1987) in the Invitrogen™ Explorer NTI program. An identity table (not shown) displaying the percentage of identical residues among the sequences ranged from 100 to 97% for all pairwise comparisons in the total alignment. When sequences were grouped by collection site and then separately aligned within their respective group, the collection sites of Patuxent NWRR, Suwannee NWR, Fort Campbell, Ossabaw Island and Fort Rucker displayed 100 to 99% of identical residues among the sequences for all ungapped positions between the pairwise comparisons while the sites of Baton Rouge and Fort Bragg displayed 100 to 98%.

While the Invitrogen™ NTI Explorer program provided a sense of the topology of the relationship between the sequences, a statistical estimate of the reliability of the groupings was made by bootstrapping (Hall, 2001) using the PHYLIP and PAUP packages. Figure 2 provides the consensus tree derived from the PHYLIP package, Version 3.6, using SEQBOOT, DNAPARS, and CONSENSE programs and the DRAWTREE application. Bootstrap values, as derived in PAUP from 1,000 bootstrap replications with 50% majority rule consensus tree, ranged from 51 to 62%.

Interpopulational (between collection sites) sequence diversities as determined by the distance matrix program Kimura's 2-parameter genetic distance model ranged from 0 to 2.7%, with all but ten sequences having 0% genetic distance. The ten sequences that displayed nonzero genetic distance values were from Fort Rucker (4), Patuxent NWRR (3), Baton Rouge (2), and Fort Bragg (1). The intrapopulational (within collection site) sequence diversities were the following: Fort Bragg (0 to 1.8%), Fort Campbell (0%), Fort Rucker (0 to 1.8%), Suwannee NWR (0%), Ossabaw Island (0%), Patuxent NWRR (0 to 0.4%), and Baton Rouge (0 to 1.8%).

DISCUSSION

Phylogenetic analysis of the ITS2 sequenced region of *Lu. shannoni* from seven sites within the southeast U. S. does not support significant divergence among the subject populations. While the intrapopulational sequence diversity was lower than the interpopulational sequence diversity (1.8% maximum distance compared to 2.7% maximum distance) as determined by the Kimura's 2-parameter genetic distance model, all of the divergence was due to 10 specimens originating from four of the collection sites. If sand flies from a certain collection site were part of a separate population, large interpopulational genetic distances would be expected from all of the specimens collected at that site when compared to the other sites. This was not observed in this study as 32 of the 42 specimens had essentially identical sequences (0% genetic distances) while the remaining 10, with varying differences, originated from four different collection sites. The bootstrap values were generally low, indicating low confidence levels in the structuring of the clades. When all sequences were compared, no single collection site could be discerned as is evident in Figures 1 and 2. The small amount of variation observed in the sequences did not have a diagnostic distribution and were not informative in distinguishing the specimens based upon collection site. At the locations examined, there exists no significant divergence indicating that a single population of *Lu. shannoni* may exist throughout the southeastern United States.

Nine of the 12 sample specimens that separated out from the main grouping of the COI analysis (Chapter 3) were used in this ITS2 analysis (3 of the 6 Patuxent NWRR that had separated out were not used as the entire sample in the ITS2 analysis would then have been composed of possibly divergent specimens). Only 2 of the 9 specimens (1

from Fort Bragg and 1 from Patuxent NWRR) separated out from the main grouping in the ITS2 analysis as in the COI results. The other 7 specimens displayed no variation to the sequences of the major grouping as the compared genetic distances were 0%. The variation in the COI among these specimens was not observed in the ITS2 analysis.

As in the COI study, it was hypothesized that the sites of Ossabaw Island or Fort Campbell might show significant divergence based upon allopatric speciation process. The island environment of Ossabaw Island and the Appalachian mountain chain separating Fort Campbell from the other sites may serve as isolating mechanisms precipitating phylogeographic divergence. No such divergence was observed in this ITS2 analysis as all specimens from Ossabaw Island had the exact same ITS2 sequence when compared to the main grouping. Five of the 6 Fort Campbell specimens also had the same exact sequence as the main grouping; one sample had a bp difference: a deletion at position number 34 for the base Adenine.

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Figure 1. Relationship among the ITS2 sequences as represented by the sequence distance method Neighbor Joining (NJ) algorithm of Saitou and Nei (1987) as calculated in the Invitrogen™ Explorer NTI program. Scores of the crude similarity between all pairs of sequences ("Parities alignment") are shown in parentheses following each specimen number.
BG: Fort Bragg, BR: Baton Rouge, FC: Fort Campbell, FR: Fort Rucker,
OS: Ossabaw Island, SU: Suwannee NWR, PX: Patuxent NWRR, GenBk: GenBank

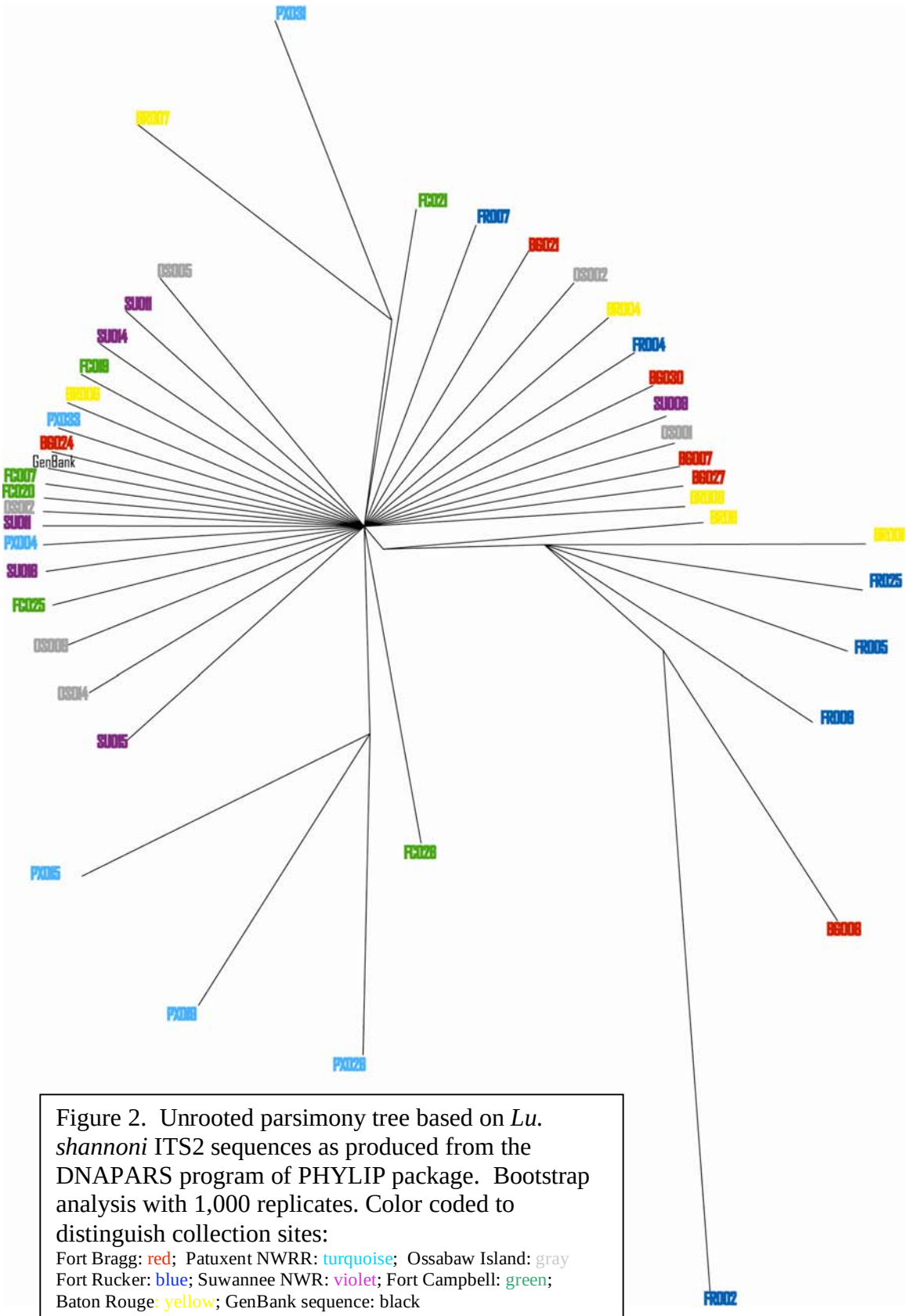

Figure 2. Unrooted parsimony tree based on *Lu. shannoni* ITS2 sequences as produced from the DNAPARS program of PHYLIP package. Bootstrap analysis with 1,000 replicates. Color coded to distinguish collection sites:
Fort Bragg: red; Patuxent NWRR: turquoise; Ossabaw Island: gray
Fort Rucker: blue; Suwannee NWR: violet; Fort Campbell: green;
Baton Rouge: yellow; GenBank sequence: black

Chapter 5

Morphological anomalies in two *Lutzomyia shannoni* specimens collected from Fort Rucker, AL and Fort Campbell, KY

INTRODUCTION

There are several reports of morphologic anomalies in sand flies (Marcondes, 1999; Gallego et al., 1994; Gallego et al., 1991; Kassem et al., 1988; Addadi and Dedet, 1977). The importance of these anomalies appears to be largely unknown as the publications focus on the physical description as opposed to discussing how the subject features affect taxonomic status of the specimens. Marcondes (1999) described seven specimens of *Lu. intermedia*, including three male specimens that possessed anomalies in the number of spines on the gonostylus. The author cited studies on other species including *Lu. bahiensis* (Sherlock, 1963) where there is such large variability on "the number of spines on style, that it is difficult to define which are anomalous specimens." The gonostylus is of major taxonomic importance as it is the terminal segment of the male genitalia known as the "claspers", which enables copulation with the female of the species. The taxonomic keys of Young and Perkins (1984) use the number of spines on the gonostylus in the first couplet to differentiate two major groupings of North American sand flies.

This chapter describes two male specimens of *Lu. shannoni* that were observed to have anomalies to the number of spines on the gonostyli. The molecular marker techniques from Chapters 3 and 4 were used to evaluate the genetics of the specimens and confirm species identification.

MATERIALS AND METHODS

A total of 778 sand flies were collected from seven widely separated locations in the southeastern United States (Baton Rouge, LA; Fort Bragg, NC; Fort Campbell, KY; Fort Rucker, AL; Ossabaw Island, GA; Patuxent NWRR, MD; and Suwannee NWR, FL) and mounted on microscope slides for identification. The identification of some specimens could not be conclusively determined to species due to absence of antennal or abdominal segments, presumably caused by damage during collection in the light trap (see Chapter 2). These specimens were excluded from both the morphometric and molecular analyses. Also excluded were two male specimens that possessed morphological anomalies. These two specimens are described and analyzed.

Fort Rucker specimen # 078:

Fort Rucker specimen # 078 (FR078) is a male specimen noted to have the asymmetrical anomaly of five spines on one gonostylus and four spines on the other. The specimen was collected in a light trap during the period of September 10 – 12, 2005. Figure 1 is a micrograph of the genitalia showing the gonostylus possessing the 5^{th} spine as indicated by the arrow. The other gonostylus has four spines, with no empty socket where a missing spine would have been attached. The specimen has long, distinct apical spurs on the ascoids of the antennal flagellomeres indicating that it is *Lu. shannoni* according to the keys of Young and Perkins (1984).

The preserved parts of the specimen not used in the slide mounting (see Chapter 2) were used in DNA extraction, amplification, and partial sequencing (see Chapters 3 and 4 for detailed methodologies) of the molecular markers COI and ITS2.

Fort Campbell specimen # 014:

Fort Campbell specimen # 014 (FC014) is a male with five rather four spines on each gonostylus as shown in Figure 2. Collection of this specimen was made in a light trap during the period of September 6 – 8, 2005. Initially this specimen could have been mistaken for *Lu. vexator* which possesses five spines on each gonostylus and was also collected at the Fort Campbell site. However, the specimen has long, distinct proximal spurs on the ascoids of the antennal flagellomeres consistent with the identification of *Lu. shannoni* (*Lu. vexator* has simple ascoids, without proximal spurs).

The preserved parts of FC014 underwent DNA extraction, amplification, and partial sequencing of the molecular markers COI and ITS2.

RESULTS

FRO78:

Figure 3 shows the relationship of FR078 COI sequence to the other Fort Rucker specimens used in the COI analysis of Chapter 3. FR078 shows the most variation, but it is not sufficient to conclude a different species. When the CO1 sequence of FR078 was compared to all the COI sequences generated from each collection site of this study (tree not shown), the sequence did not show any significant variation as it was grouped nearly in the middle of the variational range of all the sequences. Figure 4 shows the relationship of FR078 ITS2 sequence to the other Fort Rucker specimens used in the ITS2 analysis of Chapter 4. FR078 shows no significant variation of the ITS2 as compared to the other *Lu. shannoni* specimens.

FC014:

Figure 5 shows the relationship of FC014 COI sequence to the other Fort Campbell specimens used in the COI analysis of Chapter 3. FC014 does not differ significantly from the *Lu. shannoni* specimens collected at that location. Figure 6 shows the relationship of the FC014 ITS2 sequence to the other Fort Campbell specimens used in the ITS2 analysis of Chapter 4. FC014 has the exact ITS2 sequence as the other *Lu. shannoni* specimens from that location.

DISCUSSION

The two anomalous specimens presented in this chapter were identified as *Lu. shannoni* based on the following: 1) both specimens possess antennal ascoids with long, distinct proximal spurs (a near diagnostic character of *Lu. shannoni* in North America), 2) the sequences of the partial COI gene from both specimens indicated *Lu. shannoni*, and 3) the sequences of the ITS2 molecular marker from both specimens indicated *Lu. shannoni*. The anomalous features are fundamentally different from each other as FR078 possesses a fifth spine (basally located) on just one gonosylus while FC014 possesses five spines (extra spines subterminally located) on both gonostyli.

Anomalies in the number of spines on the gonostyli are not only of major taxonomic importance, but also are important biologically as the gonostyli are reproductive organs. Would these specimens have been able to successfully reproduce with female *Lu. shannoni* or were they destined to become biological "dead ends"? If successful reproduction could have taken place, would the feature have conferred an advantage, neutrality, or a disadvantage in the selection process? Other obvious questions are: What is the frequency of these anamolies in the natural population? Do certain areas show a greater prevalence than others? Are these anomalies the result of possible introgression with a 5-spine species such as *Lu. vexator*?

It is mere speculation as to how these specimens would have fared, but most likely the anomalies are disadvantageous given the low frequency of appearance in the population. If the anomalies are due to a genetic change, vice a developmental mishap, one would naturally expect disadvantageous traits to have very low frequencies. When the two anomalies are compared to the total number of male *Lu. shannoni* collected

throughout the study, a ratio of 2/281 (71/10,000) is derived. Marcondes (1999) found the observed frequency of anomalies in *Lu. intermedia* to be 128/10,000 and cited studies using other sand fly species with observed anomalies that ranged from 3/10,000 to 22/10,000. There appeared to be no aggregation of the anomalous features in any one particular region.

The species *Lu. vexator* has a range that encompasses the entire range of *Lu. shannoni* in the United States (Young and Perkins, 1984; Williams, 1991). *Lu. vexator* was collected in small numbers at five of the seven collection sites in this study including Fort Rucker and Fort Campbell (see Chapter 2). The females are believed to specifically target reptiles for blood meals (Young and Perkins, 1984; Schall, 2000; Klein et al., 1987) although suspicion has been raised as to it being a possible vector of parasites to mammals (Ostfeld et al., 2004).

Introgression in insects has been documented based on COI haplotypes (Bull et al., 2006; Hyashi et al., 2005; Garcia and Powell, 1998). Testa et al. (2002) and Marcondes et al. (1997) reported introgression of the mitochondrial cytochrome b gene in sand flies. Since the male *Lu. vexator* possesses five spines on each of the gonostyli, could the two anomalous specimens be a result of introgression between *Lu. shannoni* and *Lu. vexator*? This appears unlikely, as the COI and ITS2 sequences do not show significant differences from the other *Lu. shannoni* specimens. However, a definitive statement cannot be made since there are no GenBank-entered sequences of the COI or the ITS2 for *Lu. vexator* and therefore a comparison cannot be made. The possibility exists that *Lu. vexator* may have very similar sequences of both markers as a result of chronic introgression. Yet, a more

feasible reasoning is that the anomalies are the result of a developmental mishap or congenital defect as opposed to a genetic change such as a mutation or introgression.

Although this analysis was but a minor part of the main body of research, it validates the earlier claim that one cannot rely upon just a single taxonomic character when differentiating taxa, but rather should use a combination of morphological and molecular techniques. These subject specimens, especially FC014, could easily have been misidentified if a researcher relied solely on morphological keys.

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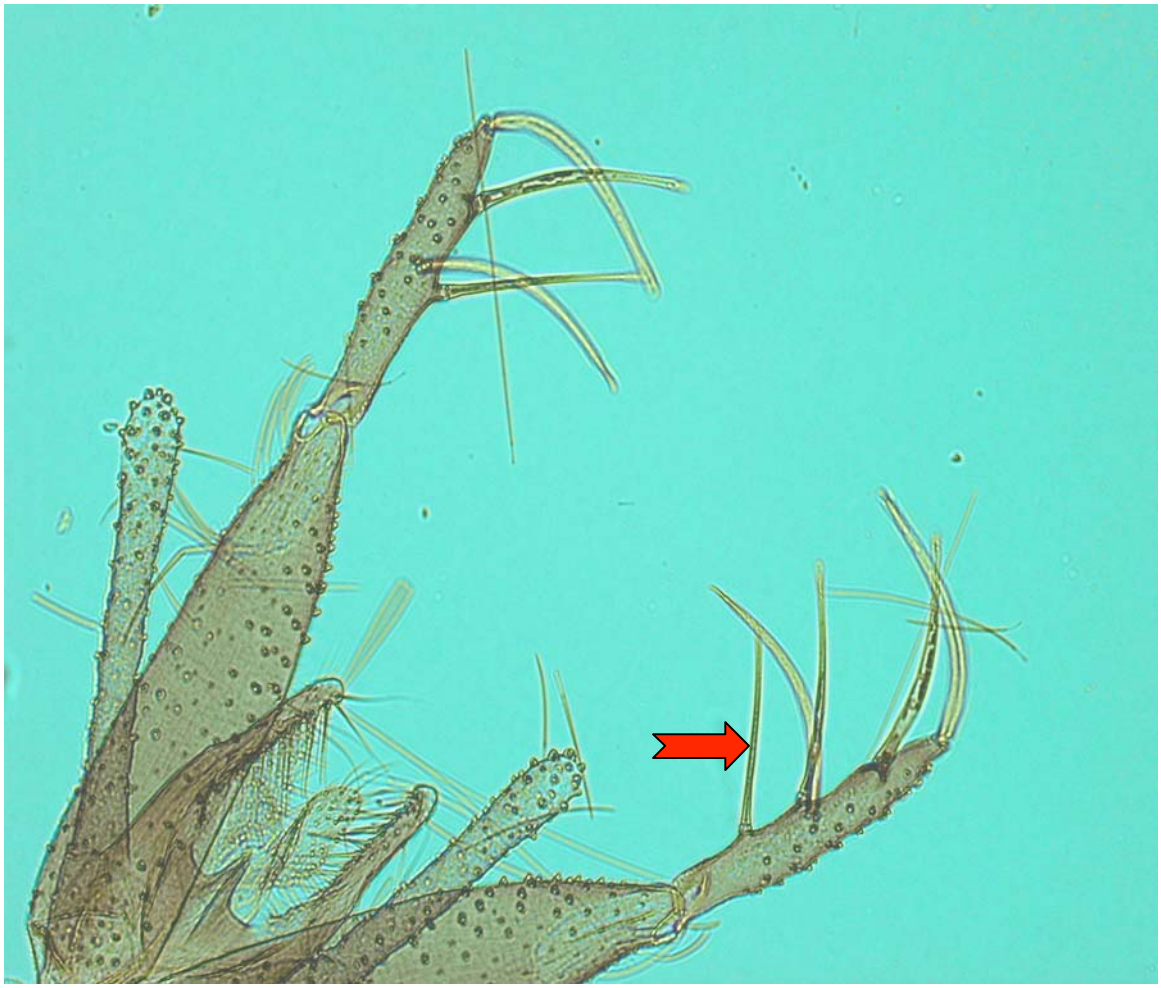

Figure 1. Micrograph of the genitalia of male specimen FR078 with arrow pointing to the 5th spine on one of the gonostyli

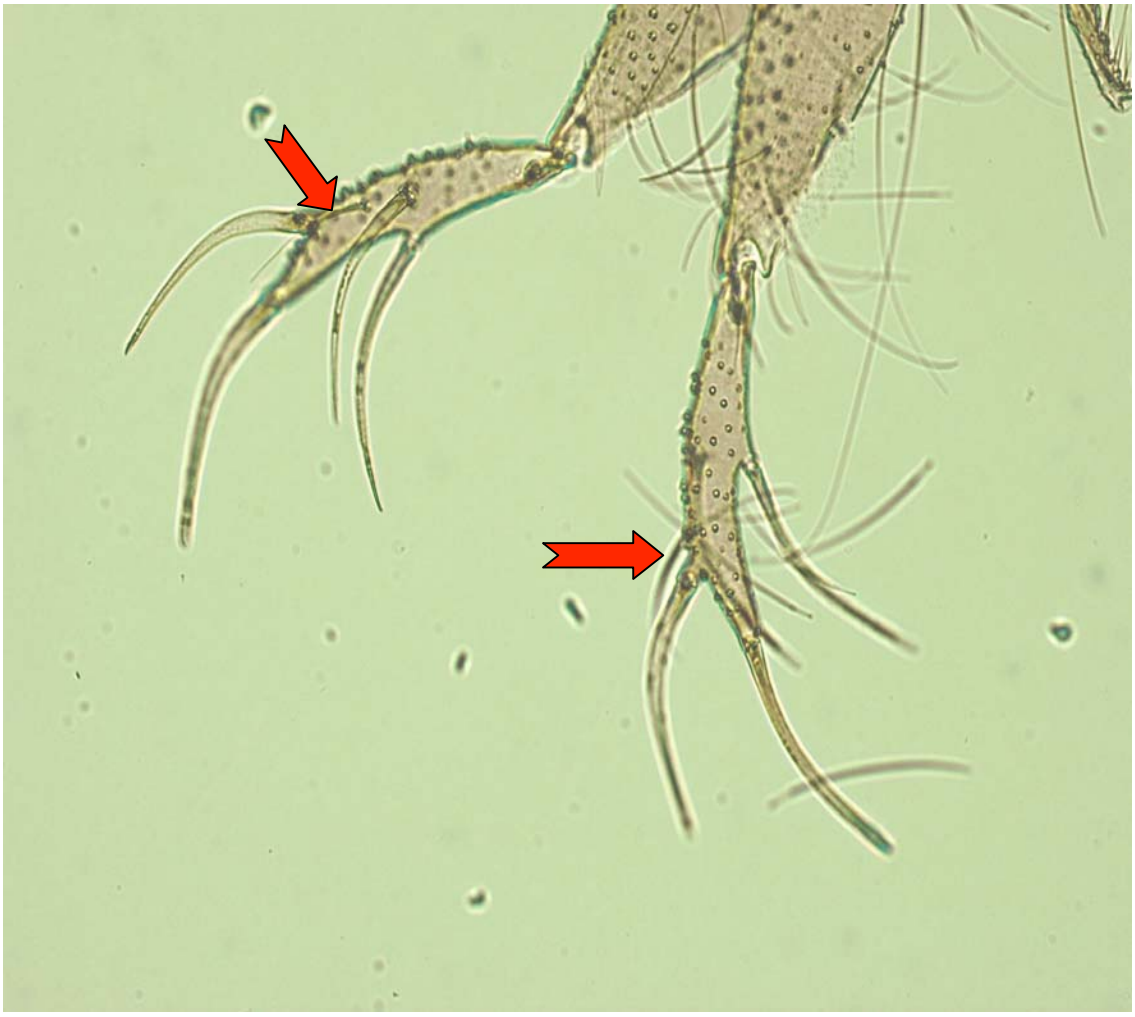

Figure 2. Micrograph of genitalia of male specimen FC014 with red arrows pointing to the 5th spine on each gonostylus

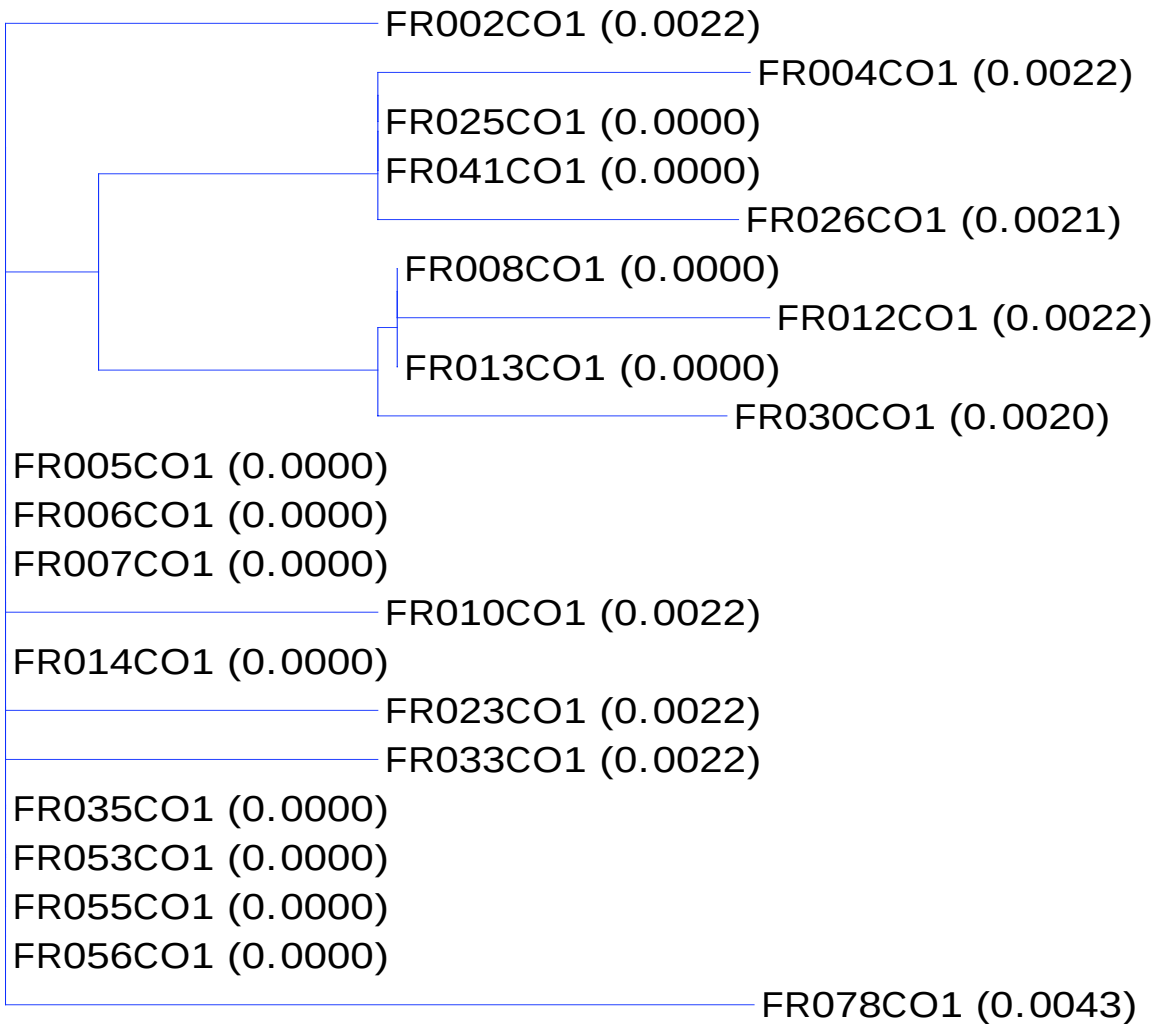

Figure 3. Relationship of CO1 sequences from Fort Rucker, AL specimens with FR078, as represented by the sequence distance method Neighbor-Joining algorithm of Saitou and Nei (1987)

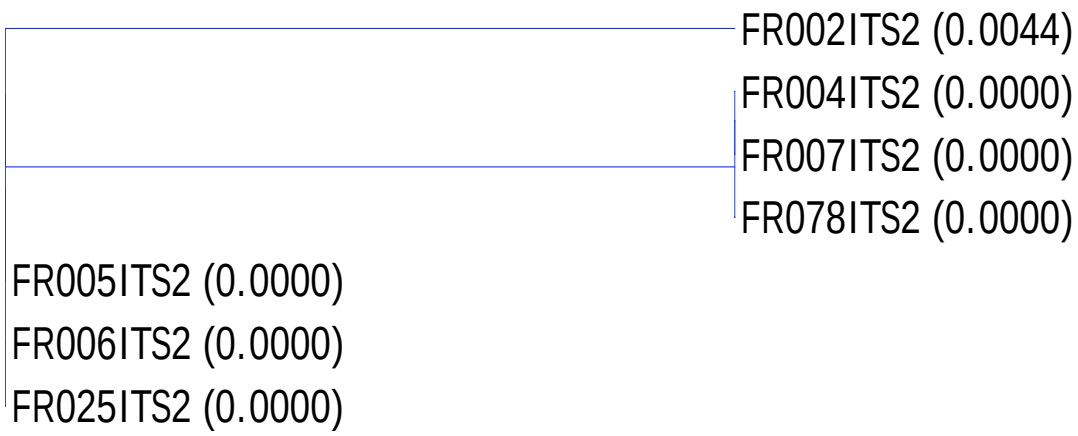

Figure 4. Relationship of ITS2 sequences from Fort Rucker, AL specimens with FR078, as represented by the sequence distance method Neighbor-Joining algorithm of Saitou and Nei (1987)

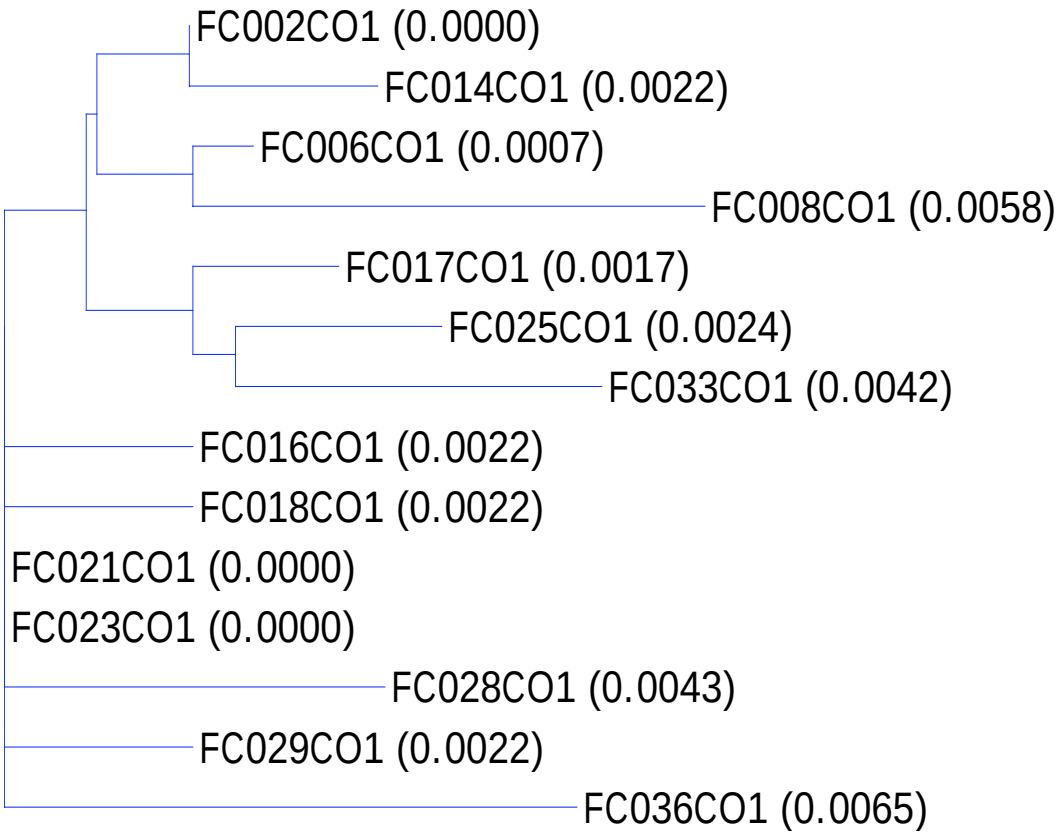

Figure 5. Relationship of CO1 sequences from Fort Campbell, KY specimens with FC014, as represented by the sequence distance method Neighbor-Joining algorithm of Saitou and Nei (1987)

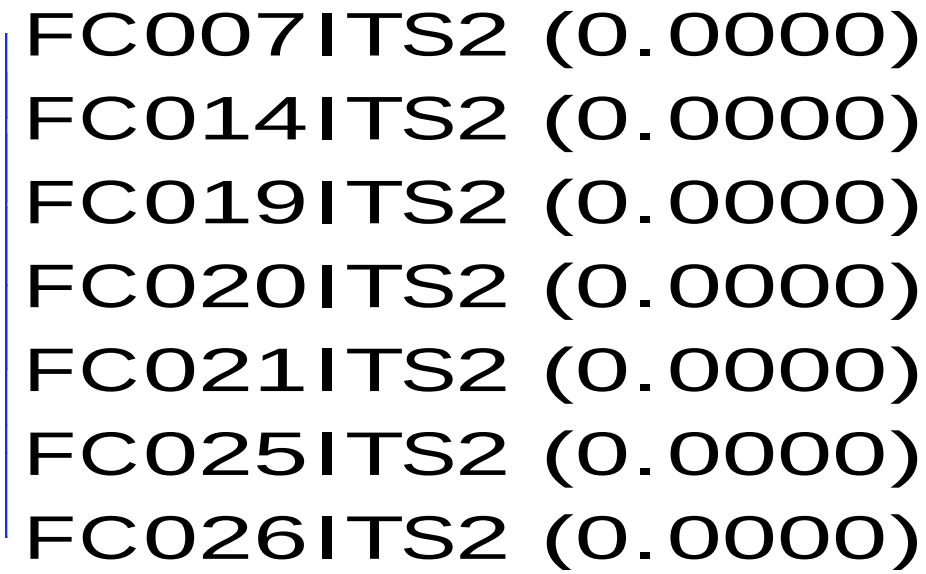

Figure 6. Relationship of ITS2 sequences from Fort Campbell, KY specimens with FC014, as represented by the sequence distance method Neighbor-Joining algorithm of Saitou and Nei (1987)

Chapter 6

Population dynamics of *Lutzomyia shannoni* at the Patuxent National Wildlife Research Refuge, Maryland from June 23, 2005 – June 15, 2006

ABSTRACT

The population dynamics of the sand fly *Lutzomyia shannoni* were examined at the Patuxent National Wildlife Research Refuge, MD from June 23, 2005 – June 15, 2006. A total of 116 (44 males, 72 females) *Lu. shannoni* were collected in 4 light traps baited with dry ice and each set at the same designated location throughout the study. All 4 traps, separated by a maximum distance of approximately 1 mile, operated simultaneously on the collection dates. The collection dates were spaced apart by near weekly intervals during the expected periods of sand fly activity. No collections occurred in December – February. The month of August was clearly the time period of peak abundance for this species as the numbers collected were significantly greater than any other month of collection. Results indicate the existence of a unimodal pattern of abundance, but a bimodal or even a trimodal pattern cannot be ruled out. Continued research is needed to compile multi-year data to confirm the temporal and prevalence patterns indicated in this study.

INTRODUCTION

In the study of any insect species, especially a potential vector, knowledge of the population dynamics is of utmost importance (Lord, 2004). A researcher needs to know the temporal sequence and seasonal abundance of a suspected vector in a given area if accurate risk predictions are to be made. Furthermore, if the insect is to be collected for laboratory rearing and experiments, personnel labor and financial resources can be maximized if one knows the most productive time frame to conduct collections. The objectives of this part of the study were to investigate the seasonal abundance and temporal patterns of adult *Lu. shannoni* at the Patuxent National Wildlife Research Refuge (NWRR), an area that is near to the northern range limit of this species.

Brinson et al. (1992) examined seasonal abundance, effects of environmental factors, vertical distribution, and diurnal resting sites of *Lu. shannoni* on Ossabaw Island, GA. Using CDC miniature light traps supplemented with dry ice, it was found that adult *Lu. shannoni* were abundant for the 4-month period of May through August with first appearance in April and disappearance in November. Interestingly, the capture in the CDC miniature light traps supplemented with dry ice yielded a sex ratio of 4.9 males to 1 female. Males are of course not hematophagous, but it is supposed that mating with the females takes place on or near the host; a light trap may attract a disproportionate number of males for the purpose of reproduction. The number of collected sand flies reached a maximum in the month of May, however there were other lesser peaks of abundance in the months of April, July, and September. Mean nightly air temperature was positively correlated with light trap catches; more than 90% of the collection occurred when mean nightly air temperatures were between 17.1º C (62.8º F) and 30.6º C (87.1º F). No adult

sand flies were collected if mean nightly air temperatures decreased beyond 10º C (50º F). The authors found no significant effect on trap catches due to moon phase or wind speed although rainfall was negatively correlated with collection numbers if the rain occurred 14 days prior to collection (presumably resulting in increased mortality to the immature stages). [de Aguiar and Soucasaux (1984) reported *Lu. shannoni* from a study site in Brazil to be the most resistant to unfavorable weather such as wind or rain when compared to other *Lutzomyia* and that conversely a new moon resulted in greatest capture of *Lu. shannoni*. It is possible that the Brazilian populations are significantly different from the North American populations and hence exhibit different behavioral patterns in response to moon phases.] Adult activity was greatest at ground level of 0.5 meter and decreased significantly with the 4- and 8-meter vertical distribution testing heights. Aspirator collections of sand flies were made at diurnal resting sites in tree cavities ranging from oaks, hickories, and magnolias. Other potential resting areas were also sampled such as ground litter, tree foliage, bark, buttresses, and cavities, but only the tree cavities produced collections. The typical sand fly-producing cavity was found to be close to the ground (less than 2 meters), small (0.2 cubic meters), and yielded a mean of five adults.

In a follow-up study to the above, Comer et al. (1994a) stated that *Lu. shannoni* on Ossabaw Island undergoes facultative diapause and that two or three generations occur in most years. The temporal sequence is given as follows: “The first generation emerges in the spring as diapause is terminated and a second occurs in mid-summer. A third generation of adults may occur at the end of the summer or in the early fall if conditions are favorable.” Developmental times per generation ranged from 8 to 10 weeks and were

presumed to be reflective of temperature differences. The facultative diapause stage is proposed to be in one of the larval instars although the authors noted that neither the overwintering sites nor the larvae could be located during the study and therefore exactly what larval instar diapauses is left open.

Comer et al. (1993) conducted an experiment to determine the forest type effect on the distribution of *Lu. shannoni* on Ossabaw Island. Ossabaw Island can be divided based upon geological formations: the Pleistocene and the Holocene. The soil associations of the formations differ to the point that past agriculture had been limited to the Pleistocene formation leaving the poorly drained soils of the Holocene portions with extant old growth forests. The authors studied three different forest types: pine, mixed hardwood, and maritime live oak forest on factors favoring the prevalence of sand flies such as distribution of tree holes and abundance of adult flies. The study also examined the prevalence of antibodies to vesicular stomatitis virus in feral swine and the major forest type where the animals were trapped and bled. *Lu. shannoni* is believed to be a vector of vesicular stomatitis (Comer et al., 1990) and perhaps the major vector on Ossabaw Island (Comer et al., 1992). Tree hole availability, sand fly abundance, and antibody prevalence in swine were cited as being significantly greater in the maritime live oak forest than either the pine or mixed hardwood forests.

The life cycle study conducted under natural conditions by Comer et al. (1994a) is in general agreement with a life cycle study under laboratory conditions by Ferro et al. (1998). With controlled conditions of temperature and humidity, the researchers observed the life cycle of *Lu. shannoni* as the following: egg stage averaging 8.5 days, first stage larval stage averaging 9.6 days, second larval stage averaging 9.2 days, third

stage larval stage averaging 11.8 days, fourth larval stage averaging 19.9 days, and pupal stage averaging 15.2 days. The longevity of the adult was found to be an average of 8.6 days (unfortunately the gender was not differentiated). Therefore, the total egg to adult period averaged 54.6 days in the laboratory compared to the field data figure of 8 to 10 weeks from Comer et al. (1994a). Cardenas et al. (1999) reported the baseline biological growth data to be 52 days under standard laboratory incubator conditions [average maximum temperature 26.8º C (80.2º F), average minimum temperature 25.5º C (77.9º F), and 94.4% relative humidity] with 9.4 weeks generation time, intrinsic rate of population increase of 0.30, and finite rate of population increment of 1.36. Ferro et al. (1998) reported that initial collections of gravid females from the field rarely resulted in retained eggs yet later generations reared in the laboratory displayed frequent retainment of eggs by the gravid females. Additionally, female survivorship was inversely proportional to eggs retained.

MATERIALS AND METHODS

The Patuxent NWRR, Maryland is located in Prince George's and Anne Arundel counties, approximately half way between Washington, D.C. and Baltimore, MD. The refuge comprises 12,450 acres bordering both sides of the Patuxent River. The Central Tract area of the refuge, an area of approximately 2,000 acres that is closed to the public, was selected as the sampling site for this study (Figure 1). The habitat of the Central Tract area can be generally characterized as forested with large open areas of grassland/meadow and numerous wetland impoundments. Buildings that serve as the administrative offices of the Patuxent NWRR and the U. S. Geological Survey along with various maintenance facilities are located within the Central Tract but comprise a small percentage of the land area.

Population dynamics of *Lu. shannoni* were monitored from June 23, 2005 – June 15, 2006. Sand flies were collected with four John W. Hock Company New Standard Miniature Light Trap Models 1012 baited with dry ice. For the most part, weekly collections occurred from June 23, 2005 – September 30, 2005 and from March 31, 2006 – June 15, 2006. There was a 2-week hiatus in data collection from July 20 – August 3, 2005. During this time period, an American Biophysics Corporation Mosquito Magnet™ was set to run for the interval at trap location #2 but unfortunately malfunctioned shortly after being set and did not collect any insects. A 10-day interval occurred from September 20 – 30 due to rainy weather. A single collection per month was conducted in October, November, and March. No collections were made during December – February.

The targeted interval of one week between collections was generally maintained although if unfavorable weather conditions such as rain or cold nightly temperatures were predicted, the traps were set up to three days before or after the 7th day interval so as to obtain optimum collection conditions. All four light traps operated simultaneously during any night of collection; there were no separate collection times when only a single or a portion of the four traps were in operation. The light traps operated three to four hours prior to sunset to three to four hours past dawn the following day. Upon retrieval, all of the collection was placed on dry ice and transported to the laboratory where the sand flies were dissected (as per protocol detailed in Chapter 2 so as to obtain samples for the morphometric and molecular analyses), slide mounted, and identified to species using the taxonomic keys in Young and Perkins (1984).

The light traps were set at designated locations that remained the same throughout the study. Figure 2 details the light trap locations within the Central Tract area of the Patuxent NWRR. The sites were selected to represent the major types of habitat within the Central Tract area although a detailed habitat characterization was not conducted as part of this study and one was not available from the offices of the Patuxent NWRR. Trap location #1, set within a few feet of an active woodchuck burrow, was on a sharp downhill, forested slope leading to a wetland area off the Patuxent River. Trap location #2 was set at the ecotone between a young deciduous stand of trees and an open, grassy field. Trap location #3 was set in a stand of beech trees estimated to be over 30 years of age with a forest floor thick in leaf litter. Trap location #4 was set in a low-lying, dense pocket of young deciduous trees with a pond located within 300 feet. Each site had evidence of frequent use by white-tailed deer, the presumed major host of *Lu. shannoni* at

the refuge based on the study of Comer et al. (1994b) from Ossabaw Island. The light traps were set approximately 4 feet from the ground. Global Positioning System coordinates were documented for the four trap locations and are shown in Figure 2.

Meteorological parameters of temperature, relative humidity, absolute humidity, and dew point were obtained from a HOBO® H8 Pro Series logger mounted at the Trap #2 location. The logger was programmed to record the meteorological parameters at intervals of two hours. The Onset Computer Corporation's BoxCar® Pro 4.0 software interfaced with the logger when the data were accessed and subsequently exported all data to Microsoft® Excel software.

RESULTS

A total of 116 sand flies were collected at the four light trap sites over the year-long study with all identified as *Lu. shannoni.* Table 1 and Figure 3 detail the collection totals for the 12 months of the study. Two females were collected in the first week of the study (June 23, 2005) and then no collections were obtained until the first week in August (although as previously explained there was a 2-week sampling hiatus in the last half of July). The month of August was clearly the period of peak abundance for this species as the numbers collected during this period accounted for 75.9% of the total collection. A Chi-square test conducted in SPSS® 12.0.1 for Windows showed significance ($p < 0.001$) when the total collection numbers of each month were compared. In 2006, adults did not appear in the light traps until the first week in June which was a collection of just a single female. By the third week of June, the final week of the study, another 13 flies (5 males and 8 females) had been collected.

The collection numbers of August – September were analyzed statistically in terms of light trap location. The Fisher's Exact Test run from the software SAS® version 9.1 for Windows did show significance ($p = 0.0281$) in terms of total collection numbers from each light trap during the 2-month period of August – September. Therefore, the number of collected specimens among the four traps was significantly different from week to week during the time when large collections were obtained. Trap locations #1 and #3 collected nearly equal numbers of sand flies and together accounted for 76.5% of the collection for the 2-month period.

The meteorological factors of temperature, dewpoint, relative humidity, and absolute humidity in relation to total collection numbers during early July – mid October is detailed in Figure 4. Preceeding the time of major collections, a period of sustained relative humidity from July 11 – 17 is noted in addition to a peak temperature of over 90º F (31.9º C) near July 27th.

DISCUSSION

The abundance data for *Lu. shannoni* at the Patuxent NWRR indicates a unimodal pattern of abundance with comparatively small numbers present in late June/July and large numbers in August diminishing to smaller numbers in September. However, the possibility that a bimodal or even a trimodal abundance pattern exists cannot be ruled out and needs to be explored in follow-up studies. While this study relied upon data collected over a year, it is emphasized that a sound determination of an insect's abundance pattern can only be made by comparing multi-year data where redundant patterns can be examined and assessed.

Figure 3 provides the basis that all of the sand flies throughout the season are of a unimodal abundance pattern consisting of two generations (bivoltine). The "tails" of a bell-shaped abundance curve (the months of June/July being one tail, September being the other, and the peak being August) are of such low numbers that collection in light traps is remote unless the trap night happened to have been of optimal conditions for activity. This would explain the absence of collected specimens for the last week in June and the month of July.

A bimodal pattern of abundance can also be interpreted from the data. The 2-week hiatus in collections during late July complicates the analysis of the data trends, but given that data collection resulted in 0 sand flies for the three weeks preceding this hiatus and only 4 specimens were collected in the first week of August, indicate that the adult abundance pattern was just beginning to increase from a baseline of near 0. Adults could emerge in relatively small numbers throughout May and June and produce eggs that

develop into adults in August. The favorable environmental conditions for development in July would "squeeze" the emergence times of the adults into one large peak of abundance as indicated by the large collection numbers obtained for that month. In this scenario, the sand fly abundance is generally the same for the two periods, it is just that May – June emergence is spread out over the 2 months with numbers so low that capture in light traps is remote. Conversely, the offspring that emerge in August are concentrated into a 3- to 4-week window that results in comparatively large collection numbers.

A trimodal pattern with two generations can also be visualized. This annual cycle would consist of two generations: a generation of relatively low abundance that emerges in June and produces offspring that emerge in August and September. The "June generation" emerges in June, reproduces, and dies out before July and hence the absence of collection results for the first two weeks of July. The eggs laid by this generation may have different developmental rates that produce adults in August and September. Lawyer and Young (1991) reported different developmental rates of egg batches in *Lu. diabolica* depending upon time of year laid and this strategy may also be used by *Lu. shannoni*. The large numbers observed in August may be a result of favorable ecological and environmental factors or the possibility that the "June generations" lays a larger percentage of faster developing eggs (emergence in August) than slower developing eggs (emergence in September). The abundant numbers of the "August generation" and the temporal division from the "June generation" indicate that there is a generational partition. In years of very favorable conditions, it is possible that up to three generations (trivoltine) take place with a trimodal prevalence pattern. Abundance peaks could occur in the late spring, mid summer, and early fall.

While unlikely, the possibility exists that the flies within each abundance group have attained a degree of reproductive isolation based on temporal separation from the other groups. Perhaps *Lu. shannoni* at the Patuxent NWRR has an obligatory diapause in one of the immature stages that prevents adult emergence until the following year? In this scenario, adults emerging in June would have little chance to reproduce with adults emerging in August since an adult has a lifespan of just under 9 days (Ferro et al., 1998). If full separation is obtained, at least two different groups can be discerned from the data: the "June group" and the "August/September group" as these groups were separated by a dearth of flies in the month of July.

The state of Maryland is very close to the northeastern extreme of *Lu. shannoni*'s range (see Figure 1 of Chapter 1) and therefore it is not surprising that the abundance survey at Patuxent NWRR would differ from what Comer et al. (1994a) and Brinson et al. (1992) observed on Ossabaw Island, GA. In those studies, significant numbers translating to peaks in abundance were found during the months of April – July. In fact, Brinson et al. (1992) reported that the greatest number of adults captured per trap night occurred in April of the first year and in May of the second year of the 2-year study. This stands in stark contrast to no sand flies collected at Patuxent NWRR until the first week of June. Overall collection numbers from both studies in any given month were significantly higher as compared to the Patuxent NWRR collections. For instance, Comer et al. (1992) reported over 70 *Lu. shannoni* collected per trap night in April, 1986 and over 40 collected per trap night in April, 1987; Brinson et al. (1992) reported 19,788 *Lu. shannoni* collected from April 1986 to December 1987 with over 99% of that total collected from 10 light traps set 3 times a week during months of activity. After

accounting for the larger number of light traps and the greater frequency of trapping, these figures still tower over the collection results obtained at Patuxent NWRR. Being located approximately 400 miles to north of Ossabaw Island, GA, Patuxent NWRR does not have the favored live oak habitat or the warmer climate and therefore simply does not have the population size.

The light trap locations were within 1 mile of each other (Figure 2) although collections were significantly different. A full and thorough habitat characterization of each trap site was not conducted and therefore it is mere speculation as to what specific habitat factors could be responsible for the increased collections at trap sites #1 and #3. With that stated, the two trap sites did have unique conditions that may have possibly resulted in greater number of sand flies present. Trap site #1 was situated within 3 or 4 feet of an active woodchuck burrow; the mammal occupant may have provided not only bloodmeals for the female sand flies but also the den and tunnels may have been ideal developmental habitat for the immature stages. Trap site #3 was situated in a relatively mature stand of beech trees with a thick covering of leaf litter on the floor of the forest. There was ample evidence that white-tailed deer "bedded" in the area as the matted-down impressions could be readily observed. (Another indication that deer used the area as a resting site was the inordinately large number of ticks that could be found at this trap location: during the setting and retrieval of the trap it was not unusual to encounter hundreds of *Amblyomma americanum* and *Dermacentor variabilis*.) The combination of conducive habitat for the immature stages (thick leaf litter forest floor) and the availability of hosts for the blood-feeding female sand flies may be responsible for the

increased numbers collected at this particular trap site location as compared to trap sites #2 and #4.

The meteorological data for the period from July 7 – October 17, 2005 (Figure 4) show that there was a spike in the temperature approximately a week and half before the onset of the observed peak of abundance. There were also two intervals of high sustained relative humidity in mid July and late July that preceded the increase in collection numbers by approximately two weeks. These factors may be of importance in stimulating the diapausing immature stage into activation and eventual emergence as adult. However, as pointed out earlier, Brinson et al. (1992) found rainfall occurring 14 days prior to collection to have a negative effect on the numbers of adult *Lu. shannoni* collected on Ossabaw Island.

As in any population dynamic or seasonal abundance study, greater accuracy is obtained if the data are compiled over many seasons or years. Abundance and temporal patterns of a population at a certain locality will inevitably change due to environmental and ecological forces (Pianka, 1983; Solbrig and Solbrig, 1979) that manifest themselves within a very short period of time. Only by accumulating multi-year surveys can a truly accurate picture be obtained as to when and in what numbers an insect will be prevalent. The findings in this study are an initial step in determining the abundance and temporal pattern of *Lu. shannoni* in the northeast corner of its range. There appear to be significant differences in the ecology of the sand flies at Patuxent NWRR when compared to the sand flies inhabiting the warmer location of Ossabaw Island, GA. The possibility exists that there are up to three generations produced at Patuxent NWRR or perhaps even two populations separated by diapause length and time of emergence.

Continued research is needed to compile multi-year data in order to confirm the temporal and spatial patterns indicated by this study during the time period of June, 2005 – June, 2006.

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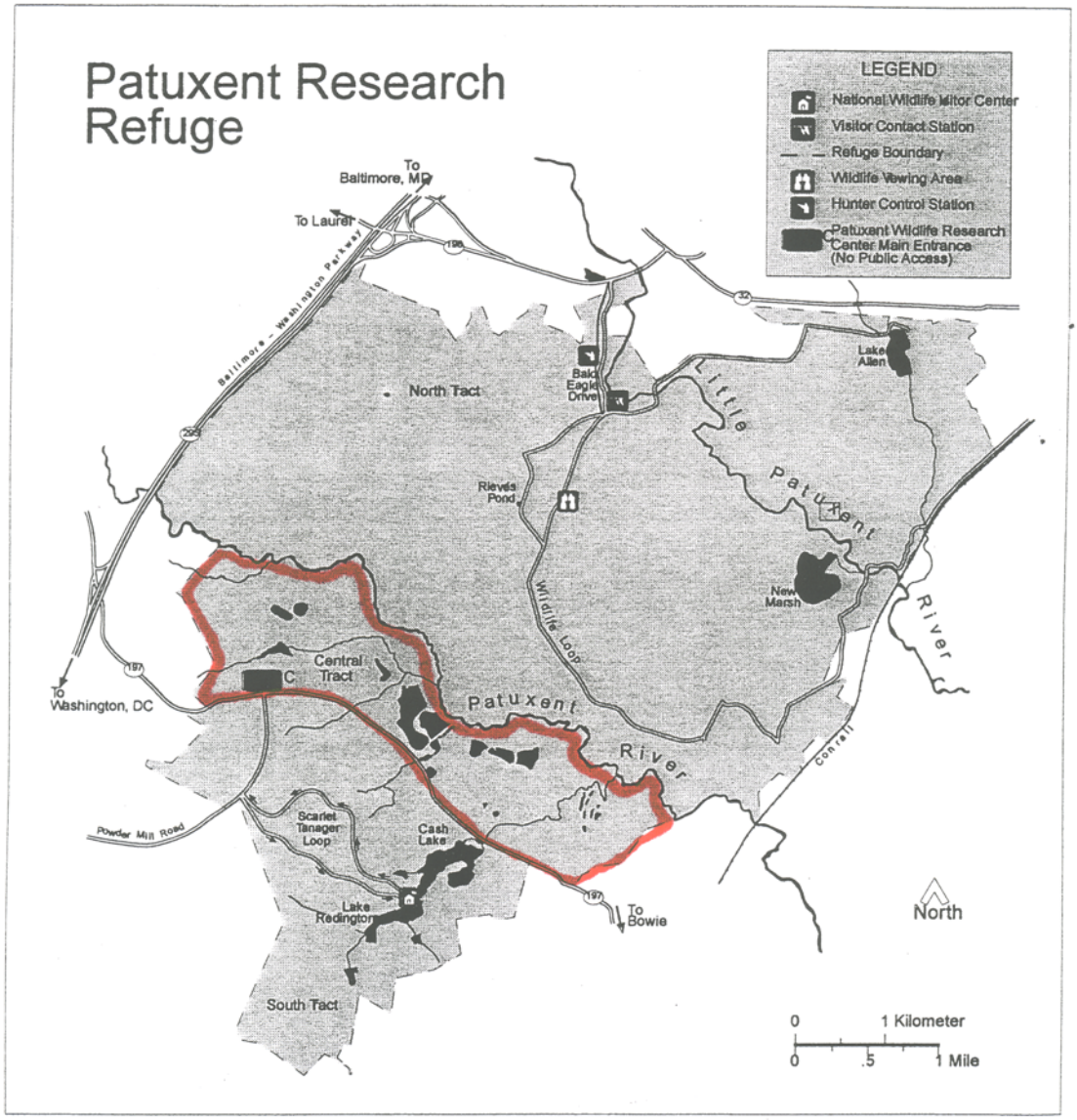

Figure 1. Map of Patuxent National Wildlife Research Refuge with approximate area of the Central Tract highlighted in red. Map provided by courtesy of Patuxent National Wildlife Research Refuge.

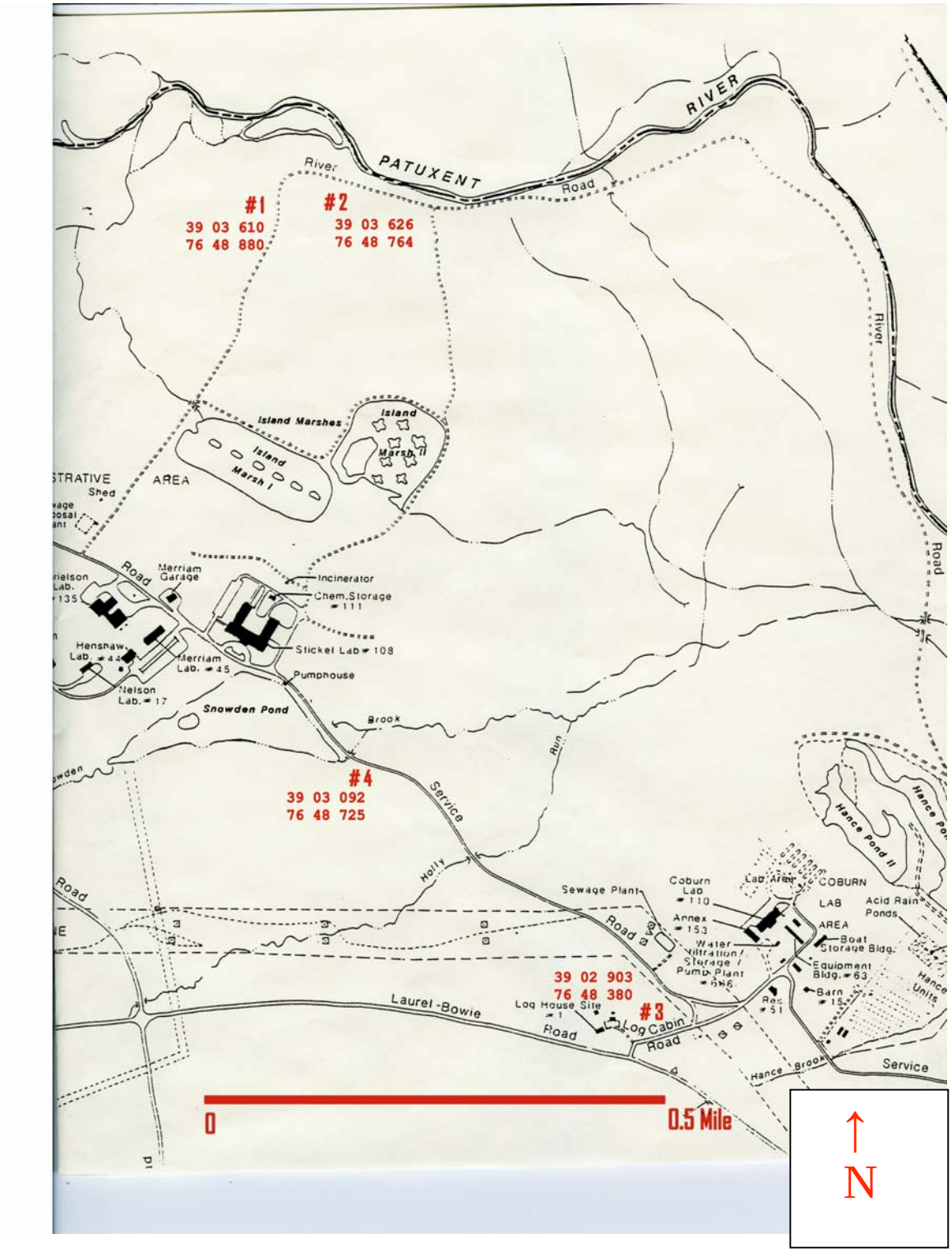

Figure 2. Map of Central Tract portion of Patuxent NWRR highlighted to show light trap locations, GPS readings of trap locations, and an approximate scale. Map provided by courtesy of the Patuxent NWRR.

COLLECTION DATE	TOTAL	Trap #1	Trap #2	Trap #3	Trap #4
23-Jun-05	2	0	2F	0	0
29-Jun	0	0	0	0	0
7-Jul	0	0	0	0	0
13-Jul	0	0	0	0	0
3-Aug	4	1F	0	1M	1M, 1F
10-Aug	13	1M	0	5M, 6F	1F
17-Aug	24	4M, 7F	1M	2M, 7F	3F
22-Aug	34	8M, 6F	3M	1M, 8F	5M, 3F
29-Aug	13	1M, 5F	0	4M, 3F	0
7-Sep	3	0	0	1M, 1F	1F
13-Sep	5	3F	1F	0	1F
20-Sep	4	0	2F	1M, 1F	0
30-Sep	0	0	0	0	0
17-Oct	0	0	0	0	0
6-Nov	0	0	0	0	0
31-Mar-06	0	0	0	0	0
9-Apr	0	0	0	0	0
15-Apr	0	0	0	0	0
21-Apr	0	0	0	0	0
28-Apr	0	0	0	0	0
4-May	0	0	0	0	0
12-May	0	0	0	0	0
18-May	0	0	0	0	0
25-May	0	0	0	0	0
1-Jun	1	0	1F	0	0
8-Jun	3	1M	0	0	2M
15-Jun	10	1M, 3F	3F	1F	1M, 1F
TOTAL	116	16M, 25F	4M, 9F	15M, 27F	9M, 11F

Table 1. Number of sand flies collected by gender, light trap location, and date at the Patuxent NWRR. M = male, F = female

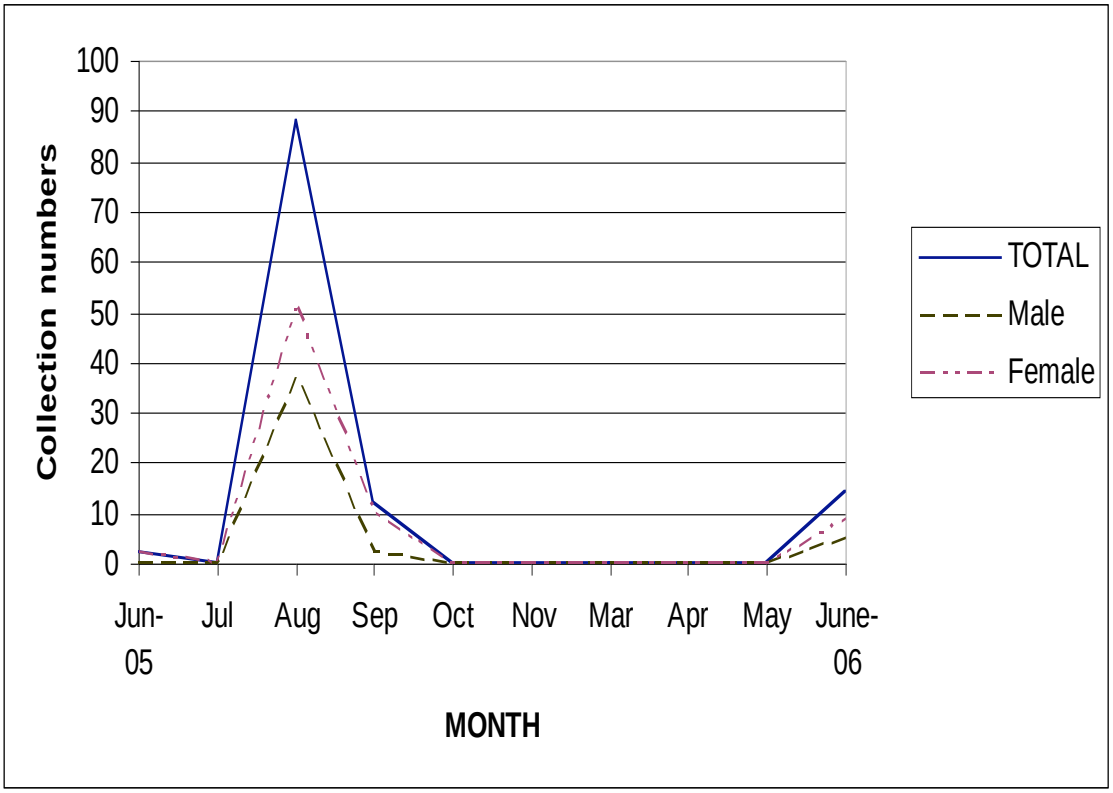

Figure 3. Graphical representation of sand fly collections by month with breakdown by gender

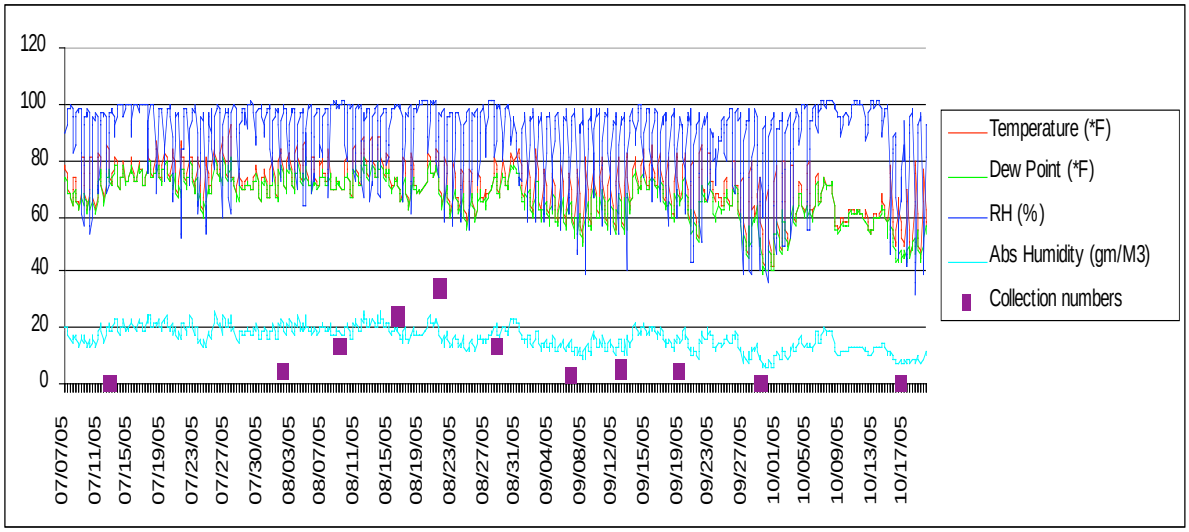

Figure 4. Graphical representation of temperature, dew point, relative humidity (RH), and absolute humidity measurements at Patuxent NWRR from July 7 – October 17, 2005 with overlay of number of sand flies collected within that period.

Chapter 7

Conclusion

CONCLUSION

This study set out with the goals of examining the variation among *Lu. shannoni* specimens collected at distant locations within the southeastern United States. The quintessential questions focused upon were: What is the degree of variation between specimens from the various collection sites? Is this degree of variation of such magnitude to indicate separate populations? Variation was measured through morphological differences (univariate and multivariate analyses) and by molecular data (COI and ITS2 markers). While the univariate analysis did show that there was variation between the specimens of the various collection sites from the characters examined, the multivariate analysis could not separate the specimens on the basis of collection site. The phenograms produced by a hierarchical cluster analysis of the ANOVA significant characters were not reproduced in the ordination or molecular methods. The variation observed in the molecular methods was generally minor, but more importantly, did not have a diagnostic distribution and was not informative in distinguishing the specimens based on collection site. Overall, the results from both methods indicate that this species of sand fly may be of one population within the southeastern United States.

A Bayesian inference of phylogeny analysis using Markov chain Monte Carlo estimation of posterior probability distributions was conducted in the computer program MRBAYES, version 3.1.2, (Huelsenbeck and Ronquist, 2000; Ronquist and Huelsenbeck, 2003) available at http://mrbayes.net. The advantage in using Bayesian inference lies in the combined data analysis that provides among-partition heterogeneity of the input data and the evolutionary process (Nylander et al., 2004). Confirmation on model use, hierarchical likelihood ratio tests, and calculating approximate Akaike

Information Criterion values of the nucleotide substitution models was obtained from the MrModeltest, version 2.2, (Nylander, 2004) at http://www.csit.fsu.edu/~nylander/mrmodeltest2/mrmodeltest2.html. The morphological data was averaged separately for both genders by collection site and given a binary code depending upon relation to the total mean from all collection sites (a "0" for any mean less than the total mean and a "1" for any mean equal to or greater than the total mean). A consensus strand from the COI and ITS2 alignments of each collection site was entered into the analysis to represent the molecular markers. When the Bayesian analysis was conducted on the morphology, COI, and ITS2 data separately, the morphology data did show Fort Rucker and Suwannee NWR grouping together with a weak 62 clade credibility value [clade credibility value is also known as a Bayesian posterior probability representing the proportion of the time each node was recovered during the stable part of the analysis (Lin and Danforth, 2004)] and the COI data grouped the collection sites of Suwannee NWR and Patuxent NWRR together with a 99 clade credibility value. The ITS2 tree showed no structuring or grouping of the collection sites. The combined analysis of using all three data types together resulted in just a single grouping (Suwannee NWR and Patuxent NWRR) with a high clade credibility value of 99. Therefore, the Bayesian analysis indicates that the Suwannee NWR and Patuxent NWRR sand flies may be more similar to each other than to flies from the other collection sites, but overall there is very little structuring (major differences) between the collection sites based on the three types of data.

The results from this study are useful to on-going research assessing the vector competency of *Lu. shannoni*. Eldridge (2004) pointed out that vector incrimination must

take place on the population level since there can be considerable intraspecific variation among arthropods that ultimately affects the vector competencies. [Eldridge (2004) defined vectorial capacity as the dynamic relationship between vectors and vertebrate hosts entailing the physiological attributes of the vector, susceptibility to infection, ability to transmit pathogens (vector competence), ecology, behavior, longevity, host preference, and abundance.] An insect that is comprised of cryptic/sibling species or have large populational variation may have inherently different ecologies, behaviors, physiologies, genetics, etc. *Lu. shannoni* in the U. S. may possess the same vector competency regardless of location as the intraspecific variation is most likely quite low in a single, panmictic population.

However, even a homogeneous population with essentially the same vector competency can exhibit different vector capabilities. For instance, the population dynamics data of *Lu. shannoni* at Patuxent NWRR indicate a single peak of large abundance occurring from mid to late August. Conversely, on Ossabaw Island there are several abundance peaks in the data from April to September (Comer et al., 1994; Brinson et al., 1992) with far greater numbers than what appear to exist at Patuxent NWRR, the northeast extreme of the species' range. Sand flies did not start appearing in the Patuxent NWRR light traps until the first week of June, a full 2 months later than what was recorded for Ossabaw Island. In the hypothetical event of a disease outbreak involving *Lu. shannoni*, the location in regards to the vector activity and abundance would be of major epidemiological importance. Therefore, even if *Lu. shannoni* at Patuxent NWRR and Ossabaw Island are biologically the same, different activity patterns can substantially increase the risk at one area compared to another.

While the results of this study indicates that there is homogeneity of the *Lu. shannoni* between the geographic areas, much future work needs to be done before such a conclusion can be definitively stated. Laboratory crossing of flies from distant locations with the production of fertile offspring would provide partial conformance to the biological species concept. Other morphological characters and molecular markers should be examined for potentially significant variation. Life stages other than the adult might have morphological features with discriminative properties. A number of collected specimens during this study were found to have parasitic mites attached to their exoskeletons. It would be interesting to study the mite species involved as different mite-host interactions may indicate population divergence. Given how little published material there is on this species, the door is wide open to examining a range of characters from the physiological, behavioral, or ecologic realms. It is also important to keep in mind that the distribution of this species spans two hemispheres and that comparison of these U. S. specimens with specimens from distant areas within the total range, i.e., Central America to Argentina, may show large degrees of variation.

An area that should be explored in greater depth is the possible temporal separation of abundance clusters as inferred from the population dynamics study conducted at Patuxent NWRR. Do other areas also exhibit indications of similar separations? Lawyer and Young (1991) documented differential rates of development for eggs of laboratory-reared *Lu. diabolica* and the consequences in regards to winter diapause duration and spring emergence patterns. A staggered emergence pattern for any species of sand fly in a temperate environment offers the advantage of optimizing resources over an unpredicatable activity season. An interesting follow-up study could compare the

variation between groups differing by the time of emergence and abundance. Are the different emergence patterns due to generations or is there a more profound separation? Perhaps the temporal separation is so complete that separate populations are actually attained in a very small geographic area? This study examined potential separation in regards to geography but the possibility exists that temporal separation is the predominate factor giving rise to variation within the species of *Lu. shannoni* inhabiting the southeastern United States.

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Appendix A

Male case summaries

Appendix A: Male Case Summaries
(Mean, Minimum, and Maximum values in microns)

location		hdlength	clypeus	interoc	labruml	palp1len
Fort Bragg	N	37.00	39.00	37.00	40.00	40.00
	Mean	368.92	93.40	104.35	245.75	49.16
	Std. Error of Mean	4.29	1.42	1.23	4.61	0.74
	Minimum	300.00	74.25	90.00	200.00	40.50
	Maximum	410.00	108.00	117.00	360.00	58.50
Fort Campbell	N	35.00	40.00	38.00	40.00	40.00
	Mean	370.29	93.38	105.04	236.50	49.11
	Std. Error of Mean	4.52	1.35	1.31	3.19	0.89
	Minimum	320.00	78.75	90.00	200.00	33.75
	Maximum	470.00	112.50	126.00	300.00	58.50
Fort Rucker	N	38.00	40.00	40.00	39.00	40.00
	Mean	361.58	92.87	103.11	247.69	48.54
	Std. Error of Mean	3.72	1.73	1.37	3.81	0.81
	Minimum	300.00	67.50	90.00	220.00	38.25
	Maximum	400.00	112.50	130.50	330.00	58.50
Ossabaw	N	36.00	40.00	39.00	40.00	40.00
	Mean	365.28	94.95	100.90	242.25	51.13
	Std. Error of Mean	3.99	1.49	1.08	3.23	0.84
	Minimum	280.00	67.50	83.25	200.00	40.50
	Maximum	400.00	112.50	114.75	300.00	63.00
Patuxent	N	40.00	40.00	40.00	40.00	40.00
	Mean	370.75	98.66	101.81	243.00	52.43
	Std. Error of Mean	3.71	1.39	1.23	2.18	0.74
	Minimum	260.00	78.75	81.00	210.00	45.00
	Maximum	410.00	119.25	119.25	290.00	67.50
Total	N	186.00	199.00	194.00	199.00	200.00
	Mean	367.37	94.66	103.01	243.02	50.07
	Std. Error of Mean	1.81	0.67	0.56	1.57	0.37
	Minimum	260.00	67.50	81.00	200.00	33.75
	Maximum	470.00	119.25	130.50	360.00	67.50

Appendix A: Male Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**palp1wid**	**palp1rat**	**palp2len**	**palp2wid**	**palp2rat**
Fort Bragg	N	40.00	40.00	40.00	40.00	40.00
	Mean	28.07	1.78	120.94	22.89	5.35
	Std. Error of Mean	0.56	0.05	1.31	0.43	0.11
	Minimum	18.00	1.33	105.75	18.00	3.93
	Maximum	36.00	2.75	139.50	31.50	6.88
Fort Campbell	N	40.00	40.00	40.00	40.00	40.00
	Mean	26.04	1.91	113.06	21.21	5.37
	Std. Error of Mean	0.46	0.05	0.88	0.29	0.07
	Minimum	22.50	1.13	101.25	18.00	4.50
	Maximum	33.75	2.27	123.75	27.00	6.38
Fort Rucker	N	40.00	40.00	40.00	40.00	40.00
	Mean	27.68	1.77	117.34	22.84	5.20
	Std. Error of Mean	0.54	0.04	1.39	0.45	0.10
	Minimum	22.50	1.33	101.25	18.00	3.79
	Maximum	33.75	2.36	139.50	31.50	6.63
Ossabaw	N	40.00	40.00	40.00	40.00	40.00
	Mean	27.68	1.86	119.19	23.29	5.19
	Std. Error of Mean	0.44	0.04	0.95	0.49	0.10
	Minimum	24.75	1.43	108.00	18.00	3.71
	Maximum	36.00	2.33	135.00	31.50	6.38
Patuxent	N	40.00	40.00	40.00	40.00	40.00
	Mean	27.39	1.94	122.34	23.01	5.38
	Std. Error of Mean	0.48	0.04	1.57	0.44	0.11
	Minimum	22.50	1.33	105.75	18.00	4.17
	Maximum	33.75	2.60	150.75	29.25	7.25
Total	N	200.00	200.00	200.00	200.00	200.00
	Mean	27.37	1.85	118.58	22.65	5.30
	Std. Error of Mean	0.23	0.02	0.60	0.19	0.04
	Minimum	18.00	1.13	101.25	18.00	3.71
	Maximum	36.00	2.75	150.75	31.50	7.25

Appendix A: Male Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**palp3len**	**palp3wid**	**palp3rat**	**palp4len**	**palp4wid**
Fort Bragg	N	40.00	40.00	40.00	40.00	40.00
	Mean	128.76	25.37	5.15	76.05	21.26
	Std. Error of Mean	1.39	0.56	0.11	1.00	0.37
	Minimum	112.50	20.25	3.60	58.50	15.75
	Maximum	146.25	33.75	6.40	87.75	27.00
Fort Campbell	N	40.00	40.00	40.00	40.00	40.00
	Mean	129.54	23.18	5.62	75.04	20.42
	Std. Error of Mean	1.25	0.31	0.08	0.80	0.29
	Minimum	112.50	20.25	3.85	67.50	15.75
	Maximum	150.75	29.25	6.70	85.50	22.50
Fort Rucker	N	40.00	40.00	40.00	40.00	40.00
	Mean	127.63	24.08	5.36	76.39	20.03
	Std. Error of Mean	1.39	0.44	0.10	0.69	0.42
	Minimum	105.75	20.25	3.57	67.50	15.75
	Maximum	148.50	33.75	6.33	85.50	31.50
Ossabaw	N	40.00	40.00	40.00	40.00	40.00
	Mean	130.39	25.48	5.20	77.34	21.38
	Std. Error of Mean	1.14	0.55	0.11	0.66	0.42
	Minimum	112.50	18.00	3.80	67.50	18.00
	Maximum	150.75	33.75	6.88	87.75	29.25
Patuxent	N	40.00	40.00	40.00	40.00	40.00
	Mean	131.01	25.93	5.13	75.66	21.09
	Std. Error of Mean	1.17	0.58	0.09	0.84	0.41
	Minimum	117.00	20.25	3.93	67.50	15.75
	Maximum	146.25	33.75	6.33	90.00	27.00
Total	N	200.00	200.00	200.00	200.00	200.00
	Mean	129.47	24.81	5.29	76.10	20.84
	Std. Error of Mean	0.57	0.23	0.05	0.36	0.17
	Minimum	105.75	18.00	3.57	58.50	15.75
	Maximum	150.75	33.75	6.88	90.00	31.50

Appendix A: Male Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**palp4rat**	**flag1len**	**flag1wid**	**flag1rat**	**flag2len**
Fort Bragg	N	40.00	40.00	40.00	40.00	40.00
	Mean	3.63	348.25	23.85	15.01	150.36
	Std. Error of Mean	0.09	5.13	0.62	0.48	1.25
	Minimum	2.17	300.00	13.50	9.78	135.00
	Maximum	5.14	460.00	33.75	25.19	166.50
Fort Campbell	N	40.00	40.00	40.00	40.00	40.00
	Mean	3.70	345.75	24.13	14.51	155.31
	Std. Error of Mean	0.06	3.63	0.46	0.29	1.14
	Minimum	3.00	290.00	20.25	8.89	141.75
	Maximum	5.00	400.00	33.75	18.77	173.25
Fort Rucker	N	40.00	40.00	40.00	40.00	40.00
	Mean	3.87	339.50	25.31	13.60	152.89
	Std. Error of Mean	0.07	3.43	0.49	0.28	1.14
	Minimum	2.57	300.00	20.25	8.89	137.25
	Maximum	5.00	390.00	33.75	16.44	173.25
Ossabaw	N	40.00	40.00	40.00	40.00	40.00
	Mean	3.66	338.25	24.75	13.86	150.58
	Std. Error of Mean	0.07	2.63	0.48	0.28	1.10
	Minimum	2.69	300.00	20.25	10.07	139.50
	Maximum	4.63	370.00	33.75	17.78	168.50
Patuxent	N	40.00	40.00	40.00	40.00	38.00
	Mean	3.63	350.25	24.08	14.68	155.84
	Std. Error of Mean	0.07	2.83	0.37	0.25	0.96
	Minimum	2.73	310.00	20.25	11.43	141.75
	Maximum	4.29	400.00	31.50	18.77	168.75
Total	N	200.00	200.00	200.00	200.00	198.00
	Mean	3.70	344.40	24.42	14.33	152.96
	Std. Error of Mean	0.03	1.65	0.22	0.15	0.52
	Minimum	2.17	290.00	13.50	8.89	135.00
	Maximum	5.14	460.00	33.75	25.19	173.25

Appendix A: Male Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**flag2wid**	**flag2rat**	**flag3len**	**flag3wid**	**flag3rat**
Fort Bragg	N	40.00	40.00	40.00	40.00	40.00
	Mean	21.83	6.97	150.53	21.54	7.04
	Std. Error of Mean	0.33	0.14	1.45	0.27	0.13
	Minimum	15.75	5.45	135.00	18.00	5.91
	Maximum	24.75	10.00	171.00	24.75	9.50
Fort Campbell	N	40.00	40.00	40.00	40.00	40.00
	Mean	21.26	7.37	152.27	20.64	7.49
	Std. Error of Mean	0.32	0.13	1.30	0.35	0.19
	Minimum	15.75	5.82	135.00	11.25	6.00
	Maximum	27.00	9.71	168.75	22.50	13.60
Fort Rucker	N	40.00	40.00	40.00	40.00	40.00
	Mean	21.21	7.27	150.75	20.59	7.38
	Std. Error of Mean	0.35	0.11	1.10	0.30	0.11
	Minimum	18.00	5.15	137.25	18.00	5.58
	Maximum	29.25	8.63	164.25	27.00	9.00
Ossabaw	N	40.00	40.00	40.00	40.00	39.00
	Mean	21.04	7.24	148.61	20.42	7.14
	Std. Error of Mean	0.36	0.13	1.12	0.59	0.10
	Minimum	15.75	5.42	128.25	0.00	5.91
	Maximum	27.00	9.00	164.25	24.75	8.50
Patuxent	N	38.00	38.00	37.00	37.00	37.00
	Mean	21.32	7.44	152.70	21.16	7.30
	Std. Error of Mean	0.46	0.17	1.20	0.41	0.13
	Minimum	15.75	5.58	137.25	18.00	5.23
	Maximum	27.00	10.14	166.50	29.25	8.63
Total	N	198.00	198.00	197.00	197.00	196.00
	Mean	21.33	7.26	150.94	20.87	7.27
	Std. Error of Mean	0.16	0.06	0.56	0.18	0.06
	Minimum	15.75	5.15	128.25	0.00	5.23
	Maximum	29.25	10.14	171.00	29.25	13.60

Appendix A: Male Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**flag4len**	**flag4wid**	**flag4rat**	**flag5len**	**flag5wid**
Fort Bragg	N	40.00	40.00	40.00	40.00	40.00
	Mean	145.52	21.32	6.88	145.74	21.38
	Std. Error of Mean	1.34	0.29	0.13	1.19	0.29
	Minimum	126.00	18.00	5.45	130.50	18.00
	Maximum	162.00	24.75	8.75	159.75	24.75
Fort Campbell	N	40.00	40.00	40.00	40.00	40.00
	Mean	148.50	19.91	7.54	149.51	19.69
	Std. Error of Mean	1.07	0.33	0.13	1.19	0.33
	Minimum	135.00	15.75	6.20	132.75	13.50
	Maximum	162.00	22.50	9.43	162.00	22.50
Fort Rucker	N	40.00	40.00	40.00	40.00	40.00
	Mean	145.74	19.80	7.42	146.48	19.91
	Std. Error of Mean	0.94	0.27	0.12	0.90	0.26
	Minimum	135.00	18.00	6.00	135.00	18.00
	Maximum	162.00	22.50	9.00	159.75	24.75
Ossabaw	N	40.00	39.00	39.00	40.00	40.00
	Mean	143.83	20.25	7.21	144.56	19.41
	Std. Error of Mean	0.88	0.40	0.14	0.90	0.29
	Minimum	132.75	15.75	5.33	130.50	15.75
	Maximum	157.50	27.00	9.29	157.50	24.75
Patuxent	N	36.00	35.00	35.00	36.00	36.00
	Mean	146.06	20.51	7.22	147.50	20.31
	Std. Error of Mean	1.03	0.40	0.15	0.98	0.39
	Minimum	132.75	15.75	5.00	135.00	15.75
	Maximum	159.75	27.00	9.29	162.00	24.75
Total	N	196.00	194.00	194.00	196.00	196.00
	Mean	145.93	20.35	7.25	146.74	20.14
	Std. Error of Mean	0.48	0.15	0.06	0.48	0.15
	Minimum	126.00	15.75	5.00	130.50	13.50
	Maximum	162.00	27.00	9.43	162.00	24.75

Appendix A: Male Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**flag5rat**	**flag6len**	**flag6wid**	**flag6rat**	**flag7len**
Fort Bragg	N	40.00	40.00	40.00	40.00	39.00
	Mean	6.87	141.24	20.36	7.08	137.71
	Std. Error of Mean	0.11	1.21	0.41	0.20	1.42
	Minimum	5.55	126.00	11.25	5.80	105.75
	Maximum	8.75	153.00	24.75	13.00	150.75
Fort Campbell	N	40.00	40.00	39.00	39.00	39.00
	Mean	7.69	144.73	19.73	7.38	141.58
	Std. Error of Mean	0.16	1.14	0.28	0.11	1.25
	Minimum	6.50	128.25	18.00	6.00	121.50
	Maximum	10.83	157.50	24.75	8.75	157.50
Fort Rucker	N	40.00	40.00	40.00	40.00	39.00
	Mean	7.41	141.86	18.73	7.64	140.37
	Std. Error of Mean	0.11	0.87	0.28	0.12	1.01
	Minimum	5.73	130.50	15.75	5.55	128.25
	Maximum	8.88	153.00	24.75	9.57	155.25
Ossabaw	N	40.00	39.00	39.00	39.00	37.00
	Mean	7.51	139.85	18.35	7.69	138.65
	Std. Error of Mean	0.11	0.89	0.29	0.12	1.00
	Minimum	5.64	128.25	15.75	5.45	123.75
	Maximum	9.43	150.75	24.75	9.00	150.75
Patuxent	N	36.00	35.00	34.00	34.00	33.00
	Mean	7.34	143.55	19.65	7.39	142.02
	Std. Error of Mean	0.13	1.06	0.37	0.14	1.04
	Minimum	5.45	126.00	15.75	5.60	126.00
	Maximum	8.86	157.50	24.75	9.14	155.25
Total	N	196.00	194.00	192.00	192.00	187.00
	Mean	7.36	142.23	19.36	7.44	140.02
	Std. Error of Mean	0.06	0.48	0.16	0.07	0.53
	Minimum	5.45	126.00	11.25	5.45	105.75
	Maximum	10.83	157.50	24.75	13.00	157.50

Appendix A: Male Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**flag7wid**	**flag7rat**	**r5l**	**wingwid**	**alpha**
Fort Bragg	N	39.00	39.00	39.00	38.00	39.00
	Mean	19.50	7.16	1252.31	648.42	488.21
	Std. Error of Mean	0.38	0.15	9.12	4.81	7.70
	Minimum	13.50	5.88	1166.00	580.00	400.00
	Maximum	22.50	10.00	1386.00	720.00	610.00
Fort Campbell	N	39.00	39.00	40.00	39.00	38.00
	Mean	19.04	7.52	1262.25	649.23	510.79
	Std. Error of Mean	0.35	0.14	8.94	4.53	8.97
	Minimum	15.75	5.17	1144.00	590.00	400.00
	Maximum	27.00	9.29	1364.00	710.00	640.00
Fort Rucker	N	39.00	39.00	40.00	40.00	40.00
	Mean	17.71	7.99	1253.45	653.50	491.75
	Std. Error of Mean	0.25	0.13	8.50	4.14	6.49
	Minimum	15.75	6.56	1144.00	600.00	410.00
	Maximum	20.25	9.43	1408.00	700.00	600.00
Ossabaw	N	37.00	37.00	37.00	37.00	38.00
	Mean	18.06	7.73	1227.84	640.00	472.37
	Std. Error of Mean	0.24	0.12	6.02	4.81	6.66
	Minimum	15.75	6.11	1144.00	570.00	360.00
	Maximum	20.25	9.43	1320.00	700.00	600.00
Patuxent	N	33.00	33.00	36.00	37.00	37.00
	Mean	18.89	7.63	1301.67	683.24	530.27
	Std. Error of Mean	0.43	0.17	11.38	6.28	8.00
	Minimum	15.75	5.09	1166.00	600.00	400.00
	Maximum	24.75	9.29	1474.00	760.00	660.00
Total	N	187.00	187.00	192.00	191.00	192.00
	Mean	18.64	7.60	1259.16	654.76	498.39
	Std. Error of Mean	0.16	0.07	4.30	2.43	3.65
	Minimum	13.50	5.09	1144.00	570.00	360.00
	Maximum	27.00	10.00	1474.00	760.00	660.00

Appendix A: Male Case Summaries
(Mean, Minimum, and Maximum values in microns)

location		delta	beta	gamma	femur	tibia
Fort Bragg	N	39.00	39.00	39.00	40.00	39.00
	Mean	141.79	304.36	243.59	891.55	1945.03
	Std. Error of Mean	5.09	5.52	4.21	11.76	15.83
	Minimum	90.00	200.00	200.00	616.00	1716.00
	Maximum	220.00	380.00	300.00	1034.00	2112.00
Fort Campbell	N	39.00	39.00	39.00	39.00	40.00
	Mean	165.64	293.33	226.67	895.79	1943.70
	Std. Error of Mean	6.89	5.11	4.46	10.54	17.12
	Minimum	60.00	210.00	170.00	748.00	1738.00
	Maximum	270.00	350.00	280.00	1056.00	2112.00
Fort Rucker	N	40.00	40.00	40.00	36.00	40.00
	Mean	162.00	295.50	234.25	889.78	1934.90
	Std. Error of Mean	6.04	5.59	4.86	8.52	16.01
	Minimum	60.00	220.00	180.00	770.00	1716.00
	Maximum	260.00	350.00	300.00	990.00	2090.00
Ossabaw	N	38.00	39.00	39.00	35.00	37.00
	Mean	145.00	297.95	225.13	834.11	1813.51
	Std. Error of Mean	6.17	4.63	4.64	7.07	20.91
	Minimum	50.00	230.00	150.00	770.00	1430.00
	Maximum	200.00	380.00	280.00	902.00	2068.00
Patuxent	N	37.00	37.00	37.00	35.00	36.00
	Mean	169.46	309.19	232.70	846.06	1916.44
	Std. Error of Mean	7.34	4.34	4.85	9.47	15.58
	Minimum	60.00	250.00	170.00	704.00	1716.00
	Maximum	320.00	360.00	280.00	946.00	2112.00
Total	N	193.00	194.00	194.00	185.00	192.00
	Mean	156.74	299.95	232.47	872.63	1911.94
	Std. Error of Mean	2.92	2.29	2.09	4.73	8.39
	Minimum	50.00	200.00	150.00	616.00	1430.00
	Maximum	320.00	380.00	300.00	1056.00	2112.00

Appendix A: Male Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**gstyllen**	**gstylwid**	**gstylrat**	**gcxlen**	**gcxwid**
Fort Bragg	N	40.00	40.00	40.00	40.00	40.00
	Mean	195.41	40.44	5.01	359.75	92.00
	Std. Error of Mean	1.56	1.26	0.15	3.44	1.48
	Minimum	157.50	27.00	2.89	320.00	80.00
	Maximum	213.75	65.25	7.67	420.00	120.00
Fort Campbell	N	40.00	40.00	40.00	40.00	40.00
	Mean	191.76	37.58	5.21	354.75	92.25
	Std. Error of Mean	1.98	0.92	0.12	4.28	1.27
	Minimum	139.50	27.00	3.41	260.00	80.00
	Maximum	213.75	49.50	6.67	400.00	110.00
Fort Rucker	N	40.00	40.00	40.00	40.00	40.00
	Mean	196.20	39.60	5.04	353.50	93.50
	Std. Error of Mean	1.20	0.82	0.10	3.39	1.32
	Minimum	184.50	27.00	4.09	300.00	80.00
	Maximum	213.75	49.50	6.92	390.00	120.00
Ossabaw	N	40.00	40.00	40.00	40.00	40.00
	Mean	191.48	41.40	4.75	340.75	92.00
	Std. Error of Mean	1.10	1.10	0.12	3.50	1.09
	Minimum	180.00	31.50	3.07	300.00	80.00
	Maximum	202.50	63.00	6.36	380.00	100.00
Patuxent	N	40.00	40.00	40.00	40.00	40.00
	Mean	193.67	44.89	4.38	358.75	96.25
	Std. Error of Mean	1.04	0.94	0.09	2.91	0.93
	Minimum	180.00	36.00	3.38	320.00	90.00
	Maximum	202.50	58.50	5.50	390.00	110.00
Total	N	200.00	200.00	200.00	200.00	200.00
	Mean	193.70	40.78	4.88	353.50	93.20
	Std. Error of Mean	0.64	0.48	0.06	1.64	0.56
	Minimum	139.50	27.00	2.89	260.00	80.00
	Maximum	213.75	65.25	7.67	420.00	120.00

Appendix A: Male Case Summaries (Mean, Minimum, and Maximum values in microns)					
location		**gcxrat**	**latlbl**	**latlbw**	**latlbrat**
Fort Bragg	N	40.00	40.00	40.00	40.00
	Mean	3.95	322.00	34.43	9.56
	Std. Error of Mean	0.07	3.65	0.84	0.24
	Minimum	2.92	270.00	22.50	6.67
	Maximum	5.00	360.00	45.00	13.33
Fort Campbell	N	40.00	40.00	40.00	40.00
	Mean	3.87	325.00	35.21	9.47
	Std. Error of Mean	0.06	3.67	0.86	0.28
	Minimum	3.18	280.00	22.50	6.22
	Maximum	4.63	380.00	45.00	14.67
Fort Rucker	N	40.00	40.00	40.00	40.00
	Mean	3.81	325.75	33.36	9.92
	Std. Error of Mean	0.07	3.08	0.70	0.22
	Minimum	2.50	290.00	24.75	7.25
	Maximum	4.50	370.00	42.75	13.33
Ossabaw	N	40.00	40.00	40.00	40.00
	Mean	3.72	323.50	34.82	9.51
	Std. Error of Mean	0.05	2.99	0.78	0.27
	Minimum	3.20	290.00	22.50	7.02
	Maximum	4.63	360.00	47.25	15.56
Patuxent	N	40.00	40.00	40.00	40.00
	Mean	3.74	318.25	37.01	8.71
	Std. Error of Mean	0.04	3.56	0.76	0.17
	Minimum	3.18	280.00	27.00	6.67
	Maximum	4.11	390.00	49.50	11.48
Total	N	200.00	200.00	200.00	200.00
	Mean	3.82	322.90	34.97	9.44
	Std. Error of Mean	0.03	1.52	0.36	0.11
	Minimum	2.50	270.00	22.50	6.22
	Maximum	5.00	390.00	49.50	15.56

Appendix B

Case summaries of female specimens

Appendix B: Female Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**hdlength**	**clypeus**	**interoc**	**labruml**	**palp1len**
Fort Bragg	N	39.00	40.00	39.00	40.00	40.00
	Mean	425.90	127.18	120.06	352.00	56.08
	Std. Error of Mean	3.40	1.71	1.36	3.25	1.12
	Minimum	380.00	96.75	99.00	300.00	45.00
	Maximum	460.00	146.25	135.00	400.00	72.00
Fort Campbell	N	37.00	40.00	39.00	40.00	40.00
	Mean	407.84	122.85	118.90	332.25	55.07
	Std. Error of Mean	3.76	1.80	1.69	3.62	0.70
	Minimum	340.00	85.50	67.50	300.00	45.00
	Maximum	450.00	146.25	135.00	400.00	67.50
Fort Rucker	N	39.00	40.00	40.00	39.00	40.00
	Mean	428.72	129.15	123.69	346.92	63.06
	Std. Error of Mean	4.41	1.56	1.41	2.73	1.00
	Minimum	380.00	103.50	112.50	310.00	49.50
	Maximum	500.00	146.25	150.75	390.00	74.25
Suwannee	N	38.00	40.00	39.00	40.00	40.00
	Mean	414.21	121.67	119.48	329.00	58.11
	Std. Error of Mean	4.18	1.48	1.24	3.06	0.91
	Minimum	360.00	90.00	96.75	300.00	45.00
	Maximum	480.00	139.50	139.50	370.00	67.50
Ossabaw	N	38.00	39.00	40.00	40.00	40.00
	Mean	406.84	125.65	113.06	337.25	61.20
	Std. Error of Mean	4.99	1.44	1.45	2.82	1.02
	Minimum	350.00	105.75	90.00	290.00	49.50
	Maximum	490.00	139.50	126.00	370.00	74.25
Patuxent	N	40.00	40.00	40.00	40.00	40.00
	Mean	415.00	133.20	116.83	341.25	62.49
	Std. Error of Mean	2.29	1.43	1.38	2.64	1.00
	Minimum	390.00	117.00	74.25	300.00	51.75
	Maximum	440.00	148.50	130.50	370.00	74.25
Total	N	231.00	239.00	237.00	239.00	240.00
	Mean	416.54	126.62	118.66	339.75	59.33
	Std. Error of Mean	1.67	0.69	0.61	1.33	0.44
	Minimum	340.00	85.50	67.50	290.00	45.00
	Maximum	500.00	148.50	150.75	400.00	74.25

Appendix B: Female Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**palp1wid**	**palp1rat**	**palp2len**	**palp2wid**	**palp2rat**
Fort Bragg	N	40.00	40.00	39.00	39.00	39.00
	Mean	32.91	1.72	165.06	26.83	6.22
	Std. Error of Mean	0.50	0.04	1.53	0.47	0.12
	Minimum	27.00	1.25	148.50	20.25	4.79
	Maximum	45.00	2.31	182.25	31.50	7.80
Fort Campbell	N	40.00	40.00	40.00	40.00	40.00
	Mean	30.94	1.81	159.30	24.64	6.56
	Std. Error of Mean	0.62	0.04	1.28	0.58	0.11
	Minimum	24.75	1.40	144.00	20.25	4.17
	Maximum	40.50	2.55	177.75	40.50	7.80
Fort Rucker	N	40.00	40.00	39.00	40.00	39.00
	Mean	33.92	1.89	164.77	29.19	5.79
	Std. Error of Mean	0.77	0.05	1.55	0.81	0.14
	Minimum	27.00	1.35	146.25	22.50	3.68
	Maximum	45.00	2.38	189.00	42.75	7.40
Suwannee	N	40.00	40.00	40.00	40.00	40.00
	Mean	34.88	1.68	164.87	29.31	5.68
	Std. Error of Mean	0.50	0.04	1.48	0.53	0.09
	Minimum	27.00	1.11	144.00	22.50	4.73
	Maximum	40.50	2.50	184.50	36.00	7.00
Ossabaw	N	40.00	40.00	39.00	39.00	39.00
	Mean	32.46	1.93	164.31	27.81	5.99
	Std. Error of Mean	0.65	0.06	1.25	0.57	0.11
	Minimum	22.50	1.35	146.25	22.50	4.47
	Maximum	40.50	3.10	180.00	36.00	7.00
Patuxent	N	40.00	40.00	40.00	40.00	40.00
	Mean	33.08	1.91	165.26	28.18	5.94
	Std. Error of Mean	0.62	0.04	1.43	0.53	0.12
	Minimum	24.75	1.33	139.50	22.50	4.67
	Maximum	45.00	2.45	191.25	33.75	7.80
Total	N	240.00	240.00	237.00	238.00	237.00
	Mean	33.03	1.82	163.92	27.66	6.03
	Std. Error of Mean	0.26	0.02	0.59	0.26	0.05
	Minimum	22.50	1.11	139.50	20.25	3.68
	Maximum	45.00	3.10	191.25	42.75	7.80

Appendix B: Female Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**palp3len**	**palp3wid**	**palp3rat**	**palp4len**	**palp4wid**
Fort Bragg	N	39.00	39.00	39.00	38.00	38.00
	Mean	169.44	31.79	5.38	86.80	22.68
	Std. Error of Mean	1.43	0.61	0.08	0.87	0.27
	Minimum	153.00	24.75	4.33	76.50	18.00
	Maximum	186.75	40.50	6.55	101.25	29.25
Fort Campbell	N	39.00	39.00	39.00	39.00	39.00
	Mean	162.23	30.92	5.33	83.65	21.58
	Std. Error of Mean	1.71	0.66	0.11	1.04	0.27
	Minimum	137.25	22.50	4.07	74.25	18.00
	Maximum	186.75	40.50	7.00	108.00	24.75
Fort Rucker	N	39.00	40.00	39.00	39.00	39.00
	Mean	168.00	34.43	5.02	83.94	22.96
	Std. Error of Mean	1.53	0.97	0.14	0.97	0.60
	Minimum	148.50	27.00	3.60	69.75	15.75
	Maximum	193.50	45.00	6.67	96.75	36.00
Suwannee	N	39.00	39.00	39.00	38.00	38.00
	Mean	162.58	35.71	4.65	82.48	23.92
	Std. Error of Mean	1.67	0.83	0.12	1.11	0.66
	Minimum	144.00	27.00	3.61	63.00	18.00
	Maximum	184.50	45.00	6.50	94.50	38.25
Ossabaw	N	39.00	39.00	39.00	39.00	39.00
	Mean	165.52	32.48	5.21	84.23	22.38
	Std. Error of Mean	1.45	0.76	0.13	0.92	0.48
	Minimum	148.50	22.50	3.95	74.25	18.00
	Maximum	189.00	42.75	7.20	99.00	33.75
Patuxent	N	39.00	39.00	39.00	39.00	39.00
	Mean	169.44	35.13	4.89	82.79	23.08
	Std. Error of Mean	1.25	0.70	0.09	0.80	0.45
	Minimum	153.00	27.00	3.75	67.50	15.75
	Maximum	182.25	45.00	6.00	90.00	31.50
Total	N	234.00	235.00	234.00	232.00	232.00
	Mean	166.20	33.41	5.08	83.98	22.76
	Std. Error of Mean	0.64	0.33	0.05	0.40	0.20
	Minimum	137.25	22.50	3.60	63.00	15.75
	Maximum	193.50	45.00	7.20	108.00	38.25

Appendix B: Female Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**palp4rat**	**flag1len**	**flag1wid**	**flag1rat**	**flag2len**
Fort Bragg	N	38.00	40.00	40.00	40.00	40.00
	Mean	3.85	324.75	25.03	13.24	136.86
	Std. Error of Mean	0.07	3.94	0.54	0.36	1.04
	Minimum	2.92	270.00	20.25	8.57	126.00
	Maximum	5.25	400.00	33.75	19.75	150.75
Fort Campbell	N	39.00	40.00	40.00	40.00	40.00
	Mean	3.90	312.25	26.38	12.09	139.16
	Std. Error of Mean	0.06	4.04	0.62	0.32	1.24
	Minimum	3.18	230.00	22.50	7.30	123.75
	Maximum	4.80	350.00	33.75	15.11	157.50
Fort Rucker	N	39.00	40.00	40.00	40.00	40.00
	Mean	3.73	319.50	28.69	11.44	140.23
	Std. Error of Mean	0.09	3.92	0.76	0.34	1.00
	Minimum	2.44	260.00	20.25	6.67	126.00
	Maximum	5.00	360.00	45.00	17.78	153.00
Suwannee	N	38.00	40.00	40.00	40.00	40.00
	Mean	3.53	320.25	28.52	11.40	141.08
	Std. Error of Mean	0.09	2.83	0.58	0.24	1.13
	Minimum	2.29	290.00	22.50	7.02	130.50
	Maximum	4.44	360.00	42.75	14.55	159.75
Ossabaw	N	39.00	40.00	40.00	40.00	40.00
	Mean	3.82	311.75	26.44	12.08	136.18
	Std. Error of Mean	0.08	2.93	0.62	0.34	1.13
	Minimum	2.67	280.00	15.75	8.33	119.25
	Maximum	5.50	350.00	36.00	18.41	148.50
Patuxent	N	39.00	40.00	40.00	40.00	40.00
	Mean	3.64	319.50	26.83	12.11	138.99
	Std. Error of Mean	0.08	2.75	0.54	0.27	0.93
	Minimum	2.57	280.00	18.00	8.89	128.25
	Maximum	5.71	360.00	33.75	15.56	150.75
Total	N	232.00	240.00	240.00	240.00	240.00
	Mean	3.74	318.00	26.98	12.06	138.75
	Std. Error of Mean	0.03	1.42	0.26	0.13	0.45
	Minimum	2.29	230.00	15.75	6.67	119.25
	Maximum	5.71	400.00	45.00	19.75	159.75

Appendix B: Female Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**flag2wid**	**flag2rat**	**flag3len**	**flag3wid**	**flag3rat**
Fort Bragg	N	40.00	40.00	40.00	40.00	40.00
	Mean	22.89	6.02	135.39	22.56	6.04
	Std. Error of Mean	0.31	0.10	1.15	0.26	0.09
	Minimum	18.00	4.46	123.75	18.00	5.00
	Maximum	29.25	7.88	157.50	27.00	7.50
Fort Campbell	N	40.00	40.00	40.00	40.00	40.00
	Mean	22.05	6.35	137.14	20.93	6.57
	Std. Error of Mean	0.29	0.09	1.30	0.20	0.07
	Minimum	18.00	5.00	121.50	18.00	5.90
	Maximum	27.00	7.44	157.50	22.50	7.67
Fort Rucker	N	40.00	40.00	40.00	40.00	40.00
	Mean	23.01	6.17	139.56	22.33	6.31
	Std. Error of Mean	0.41	0.11	1.10	0.38	0.11
	Minimum	18.00	4.71	126.00	18.00	4.75
	Maximum	31.50	8.00	157.50	29.25	7.38
Suwannee	N	40.00	40.00	40.00	40.00	40.00
	Mean	23.40	6.12	141.30	22.84	6.31
	Std. Error of Mean	0.48	0.12	1.08	0.51	0.15
	Minimum	18.00	4.21	128.25	18.00	4.29
	Maximum	31.50	7.88	155.25	31.50	8.63
Ossabaw	N	40.00	40.00	40.00	40.00	40.00
	Mean	22.89	6.04	135.17	22.22	6.14
	Std. Error of Mean	0.53	0.11	1.08	0.40	0.09
	Minimum	18.00	3.53	117.00	18.00	4.13
	Maximum	38.25	7.13	146.25	33.75	7.50
Patuxent	N	40.00	40.00	40.00	40.00	40.00
	Mean	21.83	6.42	138.09	21.54	6.46
	Std. Error of Mean	0.33	0.10	0.93	0.29	0.10
	Minimum	15.75	5.08	128.25	18.00	5.36
	Maximum	27.00	8.14	148.50	24.75	7.88
Total	N	240.00	240.00	240.00	240.00	240.00
	Mean	22.68	6.19	137.78	22.07	6.30
	Std. Error of Mean	0.17	0.04	0.47	0.15	0.04
	Minimum	15.75	3.53	117.00	18.00	4.13
	Maximum	38.25	8.14	157.50	33.75	8.63

Appendix B: Female Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**flag4len**	**flag4wid**	**flag4rat**	**flag5len**	**flag5wid**
Fort Bragg	N	40.00	40.00	40.00	40.00	40.00
	Mean	132.30	21.94	6.07	133.76	21.54
	Std. Error of Mean	0.98	0.26	0.10	1.01	0.24
	Minimum	123.75	18.00	5.09	121.50	18.00
	Maximum	146.25	24.75	7.75	157.50	24.75
Fort Campbell	N	40.00	40.00	40.00	39.00	39.00
	Mean	134.21	20.76	6.49	134.94	20.77
	Std. Error of Mean	1.26	0.22	0.08	1.20	0.25
	Minimum	119.25	18.00	5.70	121.50	18.00
	Maximum	153.00	24.75	7.88	150.75	24.75
Fort Rucker	N	40.00	40.00	40.00	40.00	40.00
	Mean	135.62	21.60	6.35	136.74	21.26
	Std. Error of Mean	1.00	0.41	0.11	1.08	0.43
	Minimum	123.75	18.00	4.75	119.25	18.00
	Maximum	153.00	29.25	7.75	157.50	31.50
Suwannee	N	40.00	40.00	40.00	40.00	40.00
	Mean	136.91	21.71	6.39	139.11	21.66
	Std. Error of Mean	0.93	0.38	0.12	0.94	0.47
	Minimum	126.00	18.00	4.83	128.25	18.00
	Maximum	150.75	27.00	8.25	153.00	29.25
Ossabaw	N	40.00	40.00	40.00	40.00	40.00
	Mean	130.16	21.32	6.18	130.73	20.87
	Std. Error of Mean	1.04	0.46	0.10	1.00	0.32
	Minimum	114.75	18.00	3.75	117.00	18.00
	Maximum	141.75	36.00	7.63	139.50	27.00
Patuxent	N	40.00	40.00	40.00	40.00	40.00
	Mean	133.54	21.04	6.39	134.78	20.93
	Std. Error of Mean	0.98	0.29	0.10	1.01	0.31
	Minimum	117.00	18.00	4.83	112.50	15.75
	Maximum	144.00	27.00	7.50	144.00	24.75
Total	N	240.00	240.00	240.00	239.00	239.00
	Mean	133.79	21.39	6.31	135.01	21.17
	Std. Error of Mean	0.44	0.14	0.04	0.45	0.14
	Minimum	114.75	18.00	3.75	112.50	15.75
	Maximum	153.00	36.00	8.25	157.50	31.50

Appendix B: Female Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**flag5rat**	**flag6len**	**flag6wid**	**flag6rat**	**flag7len**
Fort Bragg	N	40.00	40.00	40.00	40.00	35.00
	Mean	6.24	129.99	21.54	6.09	129.02
	Std. Error of Mean	0.08	1.13	0.30	0.12	1.32
	Minimum	4.91	112.50	18.00	4.67	108.00
	Maximum	7.50	150.75	27.00	7.75	153.00
Fort Campbell	N	39.00	39.00	39.00	39.00	39.00
	Mean	6.52	131.54	20.19	6.54	129.92
	Std. Error of Mean	0.07	1.15	0.27	0.07	1.10
	Minimum	5.80	117.00	18.00	5.45	112.50
	Maximum	7.75	146.25	24.75	7.50	144.00
Fort Rucker	N	40.00	39.00	40.00	39.00	35.00
	Mean	6.51	133.62	20.98	6.47	132.17
	Std. Error of Mean	0.12	1.04	0.46	0.14	1.18
	Minimum	4.50	121.50	18.00	4.43	112.50
	Maximum	7.63	150.75	31.50	8.38	150.75
Suwannee	N	40.00	40.00	40.00	40.00	35.00
	Mean	6.54	135.56	20.64	6.65	135.51
	Std. Error of Mean	0.14	1.06	0.39	0.12	1.01
	Minimum	4.46	123.75	18.00	4.75	123.75
	Maximum	8.25	153.00	27.00	8.13	148.50
Ossabaw	N	40.00	40.00	40.00	40.00	37.00
	Mean	6.31	125.21	20.31	6.21	126.24
	Std. Error of Mean	0.09	1.56	0.31	0.11	1.21
	Minimum	5.00	78.75	15.75	4.38	108.00
	Maximum	7.25	146.25	24.75	7.86	141.75
Patuxent	N	40.00	40.00	40.00	40.00	37.00
	Mean	6.50	130.84	19.69	6.70	130.74
	Std. Error of Mean	0.12	0.88	0.26	0.11	0.95
	Minimum	4.55	112.50	15.75	5.00	117.00
	Maximum	8.71	141.75	22.50	8.29	139.50
Total	N	239.00	238.00	239.00	238.00	218.00
	Mean	6.44	131.11	20.56	6.44	130.55
	Std. Error of Mean	0.04	0.51	0.14	0.05	0.50
	Minimum	4.46	78.75	15.75	4.38	108.00
	Maximum	8.71	153.00	31.50	8.38	153.00

Appendix B: Female Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**flag7wid**	**flag7rat**	**r5l**	**wingwid**	**alpha**
Fort Bragg	N	35.00	35.00	40.00	39.00	40.00
	Mean	21.21	6.13	1422.30	752.82	599.25
	Std. Error of Mean	0.31	0.12	12.42	5.47	8.71
	Minimum	18.00	4.80	1188.00	690.00	480.00
	Maximum	27.00	7.75	1672.00	850.00	750.00
Fort Campbell	N	39.00	39.00	38.00	37.00	38.00
	Mean	20.08	6.52	1398.16	747.03	580.00
	Std. Error of Mean	0.30	0.09	9.70	7.08	8.16
	Minimum	18.00	5.09	1276.00	660.00	490.00
	Maximum	24.75	7.63	1540.00	850.00	690.00
Fort Rucker	N	35.00	35.00	40.00	40.00	40.00
	Mean	20.19	6.63	1439.35	786.25	625.50
	Std. Error of Mean	0.41	0.13	16.06	7.82	10.48
	Minimum	15.75	5.00	1276.00	700.00	530.00
	Maximum	27.00	8.43	1672.00	940.00	780.00
Suwannee	N	35.00	35.00	39.00	39.00	38.00
	Mean	20.25	6.78	1376.97	731.03	590.79
	Std. Error of Mean	0.40	0.13	15.24	7.89	10.70
	Minimum	15.75	4.83	1232.00	640.00	460.00
	Maximum	27.00	8.57	1562.00	860.00	700.00
Ossabaw	N	37.00	37.00	39.00	40.00	40.00
	Mean	20.13	6.34	1347.64	721.25	560.75
	Std. Error of Mean	0.36	0.12	10.53	6.81	6.49
	Minimum	15.75	4.50	1210.00	620.00	480.00
	Maximum	27.00	7.75	1474.00	840.00	650.00
Patuxent	N	37.00	37.00	40.00	39.00	40.00
	Mean	19.52	6.76	1443.20	767.44	600.75
	Std. Error of Mean	0.30	0.11	9.38	5.09	7.67
	Minimum	15.75	5.20	1254.00	690.00	500.00
	Maximum	22.50	7.75	1540.00	830.00	710.00
Total	N	218.00	218.00	236.00	234.00	236.00
	Mean	20.22	6.52	1405.02	751.03	592.97
	Std. Error of Mean	0.14	0.05	5.54	3.09	3.79
	Minimum	15.75	4.50	1188.00	620.00	460.00
	Maximum	27.00	8.57	1672.00	940.00	780.00

Appendix B: Female Case Summaries (Mean, Minimum, and Maximum values in microns)						
location		**delta**	**beta**	**gamma**	**femur**	**tibia**
Fort Bragg	N	40.00	40.00	40.00	38.00	39.00
	Mean	211.75	326.00	273.50	959.32	1948.97
	Std. Error of Mean	8.14	4.98	5.88	8.19	17.61
	Minimum	100.00	250.00	210.00	836.00	1540.00
	Maximum	300.00	400.00	370.00	1078.00	2134.00
Fort Campbell	N	38.00	38.00	38.00	37.00	37.00
	Mean	212.37	328.16	266.58	918.65	1855.73
	Std. Error of Mean	6.52	3.94	5.06	11.13	23.26
	Minimum	140.00	270.00	200.00	770.00	1584.00
	Maximum	280.00	380.00	330.00	1100.00	2090.00
Fort Rucker	N	40.00	40.00	40.00	37.00	39.00
	Mean	221.00	335.50	286.25	913.89	1942.21
	Std. Error of Mean	6.85	6.48	5.51	9.82	16.66
	Minimum	150.00	280.00	200.00	726.00	1738.00
	Maximum	330.00	500.00	370.00	1034.00	2200.00
Suwannee	N	38.00	39.00	39.00	38.00	39.00
	Mean	208.95	317.18	252.56	878.84	1871.69
	Std. Error of Mean	8.18	4.70	5.37	8.17	19.82
	Minimum	100.00	250.00	200.00	770.00	1518.00
	Maximum	320.00	380.00	320.00	990.00	2200.00
Ossabaw	N	40.00	40.00	40.00	38.00	40.00
	Mean	196.75	325.50	258.25	863.21	1793.55
	Std. Error of Mean	6.65	4.57	6.04	7.58	21.99
	Minimum	140.00	280.00	170.00	770.00	1540.00
	Maximum	290.00	400.00	340.00	968.00	2090.00
Patuxent	N	40.00	40.00	40.00	36.00	36.00
	Mean	214.25	332.50	279.75	887.33	1920.72
	Std. Error of Mean	7.44	4.62	4.77	6.73	16.21
	Minimum	60.00	220.00	200.00	814.00	1540.00
	Maximum	300.00	400.00	340.00	990.00	2090.00
Total	N	236.00	237.00	237.00	224.00	230.00
	Mean	210.85	327.51	269.58	903.57	1888.27
	Std. Error of Mean	3.00	2.04	2.34	4.11	8.67
	Minimum	60.00	220.00	170.00	726.00	1518.00
	Maximum	330.00	500.00	370.00	1100.00	2200.00

Appendix B: Female Case Summaries (Mean, Minimum, and Maximum values in microns)					
location		**cercusl**	**sperml**	**spermw**	**spermrat**
Fort Bragg	N	39.00	39.00	39.00	39.00
	Mean	158.65	78.35	24.58	3.25
	Std. Error of Mean	3.13	1.16	0.66	0.08
	Minimum	119.25	63.00	18.00	1.94
	Maximum	202.50	94.50	40.50	5.00
Fort Campbell	N	40.00	40.00	40.00	40.00
	Mean	152.44	71.55	24.30	2.98
	Std. Error of Mean	2.59	0.94	0.44	0.06
	Minimum	126.00	56.25	20.25	2.31
	Maximum	191.25	78.75	31.50	3.89
Fort Rucker	N	39.00	32.00	32.00	32.00
	Mean	168.12	70.88	23.91	3.02
	Std. Error of Mean	3.62	1.29	0.61	0.09
	Minimum	123.75	56.25	18.00	2.27
	Maximum	213.75	87.75	29.25	4.13
Suwannee	N	40.00	29.00	29.00	29.00
	Mean	155.25	68.12	23.43	2.94
	Std. Error of Mean	3.27	0.84	0.47	0.07
	Minimum	123.75	60.75	18.00	2.45
	Maximum	200.25	78.75	27.00	3.88
Ossabaw	N	39.00	39.00	39.00	39.00
	Mean	164.13	69.23	23.77	2.96
	Std. Error of Mean	2.71	0.95	0.51	0.07
	Minimum	123.75	56.25	18.00	2.08
	Maximum	202.50	83.25	29.25	4.00
Patuxent	N	40.00	38.00	38.00	38.00
	Mean	164.81	69.45	25.11	2.80
	Std. Error of Mean	2.60	0.94	0.48	0.06
	Minimum	126.00	56.25	18.00	2.27
	Maximum	193.50	83.25	31.50	4.00
Total	N	237.00	217.00	217.00	217.00
	Mean	160.53	71.43	24.22	2.99
	Std. Error of Mean	1.27	0.48	0.22	0.03
	Minimum	119.25	56.25	18.00	1.94
	Maximum	213.75	94.50	40.50	5.00

Appendix C

Multiple comparisons of male data

Appendix C: Multiple comparisons of male data
*** The mean difference is significant at the .05 level**
Mean Difference in microns

Bonferroni

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
hdlength	Fort Bragg	Fort Campbell	-1.367	5.811	1.000
		Fort Rucker	7.340	5.692	1.000
		Ossabaw	3.641	5.769	1.000
		Patuxent	-1.831	5.621	1.000
	Fort Campbell	Fort Bragg	1.367	5.811	1.000
		Fort Rucker	8.707	5.774	1.000
		Ossabaw	5.008	5.850	1.000
		Patuxent	-.464	5.704	1.000
	Fort Rucker	Fort Bragg	-7.340	5.692	1.000
		Fort Campbell	-8.707	5.774	1.000
		Ossabaw	-3.699	5.732	1.000
		Patuxent	-9.171	5.583	1.000
	Ossabaw	Fort Bragg	-3.641	5.769	1.000
		Fort Campbell	-5.008	5.850	1.000
		Fort Rucker	3.699	5.732	1.000
		Patuxent	-5.472	5.662	1.000
	Patuxent	Fort Bragg	1.831	5.621	1.000
		Fort Campbell	.464	5.704	1.000
		Fort Rucker	9.171	5.583	1.000
		Ossabaw	5.472	5.662	1.000
clypeus	Fort Bragg	Fort Campbell	.029	2.105	1.000
		Fort Rucker	.535	2.105	1.000
		Ossabaw	-1.546	2.105	1.000
		Patuxent	-5.259	2.105	.133
	Fort Campbell	Fort Bragg	-.029	2.105	1.000
		Fort Rucker	.506	2.092	1.000
		Ossabaw	-1.575	2.092	1.000
		Patuxent	-5.287	2.092	.123
	Fort Rucker	Fort Bragg	-.535	2.105	1.000
		Fort Campbell	-.506	2.092	1.000
		Ossabaw	-2.081	2.092	1.000
		Patuxent	-5.794	2.092	.062
	Ossabaw	Fort Bragg	1.546	2.105	1.000
		Fort Campbell	1.575	2.092	1.000
		Fort Rucker	2.081	2.092	1.000
		Patuxent	-3.712	2.092	.775
	Patuxent	Fort Bragg	5.259	2.105	.133
		Fort Campbell	5.287	2.092	.123
		Fort Rucker	5.794	2.092	.062
		Ossabaw	3.712	2.092	.775

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
interoc	Fort Bragg	Fort Campbell	-.688	1.798	1.000
		Fort Rucker	1.245	1.776	1.000
		Ossabaw	3.448	1.787	.552
		Patuxent	2.539	1.776	1.000
	Fort Campbell	Fort Bragg	.688	1.798	1.000
		Fort Rucker	1.933	1.764	1.000
		Ossabaw	4.136	1.775	.208
		Patuxent	3.227	1.764	.689
	Fort Rucker	Fort Bragg	-1.245	1.776	1.000
		Fort Campbell	-1.933	1.764	1.000
		Ossabaw	2.202	1.752	1.000
		Patuxent	1.294	1.741	1.000
	Ossabaw	Fort Bragg	-3.448	1.787	.552
		Fort Campbell	-4.136	1.775	.208
		Fort Rucker	-2.202	1.752	1.000
		Patuxent	-.909	1.752	1.000
	Patuxent	Fort Bragg	-2.539	1.776	1.000
		Fort Campbell	-3.227	1.764	.689
		Fort Rucker	-1.294	1.741	1.000
		Ossabaw	.909	1.752	1.000
labruml	Fort Bragg	Fort Campbell	9.250	4.930	.621
		Fort Rucker	-1.942	4.962	1.000
		Ossabaw	3.500	4.930	1.000
		Patuxent	2.750	4.930	1.000
	Fort Campbell	Fort Bragg	-9.250	4.930	.621
		Fort Rucker	-11.192	4.962	.252
		Ossabaw	-5.750	4.930	1.000
		Patuxent	-6.500	4.930	1.000
	Fort Rucker	Fort Bragg	1.942	4.962	1.000
		Fort Campbell	11.192	4.962	.252
		Ossabaw	5.442	4.962	1.000
		Patuxent	4.692	4.962	1.000
	Ossabaw	Fort Bragg	-3.500	4.930	1.000
		Fort Campbell	5.750	4.930	1.000
		Fort Rucker	-5.442	4.962	1.000
		Patuxent	-.750	4.930	1.000
	Patuxent	Fort Bragg	-2.750	4.930	1.000
		Fort Campbell	6.500	4.930	1.000
		Fort Rucker	-4.692	4.962	1.000
		Ossabaw	.750	4.930	1.000
palp1len	Fort Bragg	Fort Campbell	.056	1.138	1.000
		Fort Rucker	.619	1.138	1.000
		Ossabaw	-1.969	1.138	.851
		Patuxent	-3.262(*)	1.138	.046
	Fort Campbell	Fort Bragg	-.056	1.138	1.000
		Fort Rucker	.563	1.138	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Ossabaw	-2.025	1.138	.766
		Patuxent	-3.319(*)	1.138	.039
	Fort Rucker	Fort Bragg	-.619	1.138	1.000
		Fort Campbell	-.563	1.138	1.000
		Ossabaw	-2.587	1.138	.240
		Patuxent	-3.881(*)	1.138	.008
	Ossabaw	Fort Bragg	1.969	1.138	.851
		Fort Campbell	2.025	1.138	.766
		Fort Rucker	2.587	1.138	.240
		Patuxent	-1.294	1.138	1.000
	Patuxent	Fort Bragg	3.262(*)	1.138	.046
		Fort Campbell	3.319(*)	1.138	.039
		Fort Rucker	3.881(*)	1.138	.008
		Ossabaw	1.294	1.138	1.000
palp1wid	Fort Bragg	Fort Campbell	2.025(*)	.703	.044
		Fort Rucker	.394	.703	1.000
		Ossabaw	.394	.703	1.000
		Patuxent	.675	.703	1.000
	Fort Campbell	Fort Bragg	-2.025(*)	.703	.044
		Fort Rucker	-1.631	.703	.213
		Ossabaw	-1.631	.703	.213
		Patuxent	-1.350	.703	.561
	Fort Rucker	Fort Bragg	-.394	.703	1.000
		Fort Campbell	1.631	.703	.213
		Ossabaw	.000	.703	1.000
		Patuxent	.281	.703	1.000
	Ossabaw	Fort Bragg	-.394	.703	1.000
		Fort Campbell	1.631	.703	.213
		Fort Rucker	.000	.703	1.000
		Patuxent	.281	.703	1.000
	Patuxent	Fort Bragg	-.675	.703	1.000
		Fort Campbell	1.350	.703	.561
		Fort Rucker	-.281	.703	1.000
		Ossabaw	-.281	.703	1.000
palp1rat	Fort Bragg	Fort Campbell	-.125	.060	.389
		Fort Rucker	.009	.060	1.000
		Ossabaw	-.080	.060	1.000
		Patuxent	-.153	.060	.118
	Fort Campbell	Fort Bragg	.125	.060	.389
		Fort Rucker	.134	.060	.270
		Ossabaw	.046	.060	1.000
		Patuxent	-.028	.060	1.000
	Fort Rucker	Fort Bragg	-.009	.060	1.000
		Fort Campbell	-.134	.060	.270
		Ossabaw	-.089	.060	1.000
		Patuxent	-.162	.060	.077

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Ossabaw	Fort Bragg	.080	.060	1.000
		Fort Campbell	-.046	.060	1.000
		Fort Rucker	.089	.060	1.000
		Patuxent	-.074	.060	1.000
	Patuxent	Fort Bragg	.153	.060	.118
		Fort Campbell	.028	.060	1.000
		Fort Rucker	.162	.060	.077
		Ossabaw	.074	.060	1.000
palp2len	Fort Bragg	Fort Campbell	7.875(*)	1.767	.000
		Fort Rucker	3.600	1.767	.429
		Ossabaw	1.744	1.767	1.000
		Patuxent	-1.406	1.767	1.000
	Fort Campbell	Fort Bragg	-7.875(*)	1.767	.000
		Fort Rucker	-4.275	1.767	.164
		Ossabaw	-6.131(*)	1.767	.006
		Patuxent	-9.281(*)	1.767	3.891E-06
	Fort Rucker	Fort Bragg	-3.600	1.767	.429
		Fort Campbell	4.275	1.767	.164
		Ossabaw	-1.856	1.767	1.000
		Patuxent	-5.006	1.767	.051
	Ossabaw	Fort Bragg	-1.744	1.767	1.000
		Fort Campbell	6.131(*)	1.767	.006
		Fort Rucker	1.856	1.767	1.000
		Patuxent	-3.150	1.767	.761
	Patuxent	Fort Bragg	1.406	1.767	1.000
		Fort Campbell	9.281(*)	1.767	3.891E-06
		Fort Rucker	5.006	1.767	.051
		Ossabaw	3.150	1.767	.761
palp2wid	Fort Bragg	Fort Campbell	1.688	.599	.054
		Fort Rucker	.056	.599	1.000
		Ossabaw	-.394	.599	1.000
		Patuxent	-.113	.599	1.000
	Fort Campbell	Fort Bragg	-1.688	.599	.054
		Fort Rucker	-1.631	.599	.071
		Ossabaw	-2.081(*)	.599	.006
		Patuxent	-1.800(*)	.599	.030
	Fort Rucker	Fort Bragg	-.056	.599	1.000
		Fort Campbell	1.631	.599	.071
		Ossabaw	-.450	.599	1.000
		Patuxent	-.169	.599	1.000
	Ossabaw	Fort Bragg	.394	.599	1.000
		Fort Campbell	2.081(*)	.599	.006
		Fort Rucker	.450	.599	1.000
		Patuxent	.281	.599	1.000
	Patuxent	Fort Bragg	.113	.599	1.000
		Fort Campbell	1.800(*)	.599	.030

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Rucker	.169	.599	1.000
		Ossabaw	-.281	.599	1.000
palp2rat	Fort Bragg	Fort Campbell	-.017	.139	1.000
		Fort Rucker	.149	.139	1.000
		Ossabaw	.156	.139	1.000
		Patuxent	-.030	.139	1.000
	Fort Campbell	Fort Bragg	.017	.139	1.000
		Fort Rucker	.166	.139	1.000
		Ossabaw	.172	.139	1.000
		Patuxent	-.014	.139	1.000
	Fort Rucker	Fort Bragg	-.149	.139	1.000
		Fort Campbell	-.166	.139	1.000
		Ossabaw	.007	.139	1.000
		Patuxent	-.179	.139	1.000
	Ossabaw	Fort Bragg	-.156	.139	1.000
		Fort Campbell	-.172	.139	1.000
		Fort Rucker	-.007	.139	1.000
		Patuxent	-.186	.139	1.000
	Patuxent	Fort Bragg	.030	.139	1.000
		Fort Campbell	.014	.139	1.000
		Fort Rucker	.179	.139	1.000
		Ossabaw	.186	.139	1.000
palp3len	Fort Bragg	Fort Campbell	-.787	1.799	1.000
		Fort Rucker	1.125	1.799	1.000
		Ossabaw	-1.631	1.799	1.000
		Patuxent	-2.250	1.799	1.000
	Fort Campbell	Fort Bragg	.787	1.799	1.000
		Fort Rucker	1.912	1.799	1.000
		Ossabaw	-.844	1.799	1.000
		Patuxent	-1.463	1.799	1.000
	Fort Rucker	Fort Bragg	-1.125	1.799	1.000
		Fort Campbell	-1.912	1.799	1.000
		Ossabaw	-2.756	1.799	1.000
		Patuxent	-3.375	1.799	.622
	Ossabaw	Fort Bragg	1.631	1.799	1.000
		Fort Campbell	.844	1.799	1.000
		Fort Rucker	2.756	1.799	1.000
		Patuxent	-.619	1.799	1.000
	Patuxent	Fort Bragg	2.250	1.799	1.000
		Fort Campbell	1.463	1.799	1.000
		Fort Rucker	3.375	1.799	.622
		Ossabaw	.619	1.799	1.000
palp3wid	Fort Bragg	Fort Campbell	2.194(*)	.707	.022
		Fort Rucker	1.294	.707	.690
		Ossabaw	-.113	.707	1.000
		Patuxent	-.563	.707	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Fort Campbell	Fort Bragg	-2.194(*)	.707	.022
		Fort Rucker	-.900	.707	1.000
		Ossabaw	-2.306(*)	.707	.013
		Patuxent	-2.756(*)	.707	.001
	Fort Rucker	Fort Bragg	-1.294	.707	.690
		Fort Campbell	.900	.707	1.000
		Ossabaw	-1.406	.707	.482
		Patuxent	-1.856	.707	.094
	Ossabaw	Fort Bragg	.113	.707	1.000
		Fort Campbell	2.306(*)	.707	.013
		Fort Rucker	1.406	.707	.482
		Patuxent	-.450	.707	1.000
	Patuxent	Fort Bragg	.563	.707	1.000
		Fort Campbell	2.756(*)	.707	.001
		Fort Rucker	1.856	.707	.094
		Ossabaw	.450	.707	1.000
palp3rat	Fort Bragg	Fort Campbell	-.470(*)	.141	.010
		Fort Rucker	-.208	.141	1.000
		Ossabaw	-.050	.141	1.000
		Patuxent	.028	.141	1.000
	Fort Campbell	Fort Bragg	.470(*)	.141	.010
		Fort Rucker	.262	.141	.649
		Ossabaw	.420(*)	.141	.033
		Patuxent	.498(*)	.141	.005
	Fort Rucker	Fort Bragg	.208	.141	1.000
		Fort Campbell	-.262	.141	.649
		Ossabaw	.158	.141	1.000
		Patuxent	.236	.141	.954
	Ossabaw	Fort Bragg	.050	.141	1.000
		Fort Campbell	-.420(*)	.141	.033
		Fort Rucker	-.158	.141	1.000
		Patuxent	.079	.141	1.000
	Patuxent	Fort Bragg	-.028	.141	1.000
		Fort Campbell	-.498(*)	.141	.005
		Fort Rucker	-.236	.141	.954
		Ossabaw	-.079	.141	1.000
palp4len	Fort Bragg	Fort Campbell	1.013	1.143	1.000
		Fort Rucker	-.338	1.143	1.000
		Ossabaw	-1.294	1.143	1.000
		Patuxent	.394	1.143	1.000
	Fort Campbell	Fort Bragg	-1.013	1.143	1.000
		Fort Rucker	-1.350	1.143	1.000
		Ossabaw	-2.306	1.143	.450
		Patuxent	-.619	1.143	1.000
	Fort Rucker	Fort Bragg	.338	1.143	1.000
		Fort Campbell	1.350	1.143	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Ossabaw	-.956	1.143	1.000
		Patuxent	.731	1.143	1.000
	Ossabaw	Fort Bragg	1.294	1.143	1.000
		Fort Campbell	2.306	1.143	.450
		Fort Rucker	.956	1.143	1.000
		Patuxent	1.688	1.143	1.000
	Patuxent	Fort Bragg	-.394	1.143	1.000
		Fort Campbell	.619	1.143	1.000
		Fort Rucker	-.731	1.143	1.000
		Ossabaw	-1.688	1.143	1.000
palp4wid	Fort Bragg	Fort Campbell	.844	.546	1.000
		Fort Rucker	1.238	.546	.245
		Ossabaw	-.113	.546	1.000
		Patuxent	.169	.546	1.000
	Fort Campbell	Fort Bragg	-.844	.546	1.000
		Fort Rucker	.394	.546	1.000
		Ossabaw	-.956	.546	.814
		Patuxent	-.675	.546	1.000
	Fort Rucker	Fort Bragg	-1.238	.546	.245
		Fort Campbell	-.394	.546	1.000
		Ossabaw	-1.350	.546	.143
		Patuxent	-1.069	.546	.517
	Ossabaw	Fort Bragg	.113	.546	1.000
		Fort Campbell	.956	.546	.814
		Fort Rucker	1.350	.546	.143
		Patuxent	.281	.546	1.000
	Patuxent	Fort Bragg	-.169	.546	1.000
		Fort Campbell	.675	.546	1.000
		Fort Rucker	1.069	.546	.517
		Ossabaw	-.281	.546	1.000
palp4rat	Fort Bragg	Fort Campbell	-.075	.102	1.000
		Fort Rucker	-.241	.102	.188
		Ossabaw	-.037	.102	1.000
		Patuxent	.000	.102	1.000
	Fort Campbell	Fort Bragg	.075	.102	1.000
		Fort Rucker	-.166	.102	1.000
		Ossabaw	.038	.102	1.000
		Patuxent	.075	.102	1.000
	Fort Rucker	Fort Bragg	.241	.102	.188
		Fort Campbell	.166	.102	1.000
		Ossabaw	.204	.102	.466
		Patuxent	.241	.102	.190
	Ossabaw	Fort Bragg	.037	.102	1.000
		Fort Campbell	-.038	.102	1.000
		Fort Rucker	-.204	.102	.466
		Patuxent	.037	.102	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Patuxent	Fort Bragg	.000	.102	1.000
		Fort Campbell	-.075	.102	1.000
		Fort Rucker	-.241	.102	.190
		Ossabaw	-.037	.102	1.000
flag1len	Fort Bragg	Fort Campbell	2.500	5.145	1.000
		Fort Rucker	8.750	5.145	.906
		Ossabaw	10.000	5.145	.534
		Patuxent	-2.000	5.145	1.000
	Fort Campbell	Fort Bragg	-2.500	5.145	1.000
		Fort Rucker	6.250	5.145	1.000
		Ossabaw	7.500	5.145	1.000
		Patuxent	-4.500	5.145	1.000
	Fort Rucker	Fort Bragg	-8.750	5.145	.906
		Fort Campbell	-6.250	5.145	1.000
		Ossabaw	1.250	5.145	1.000
		Patuxent	-10.750	5.145	.380
	Ossabaw	Fort Bragg	-10.000	5.145	.534
		Fort Campbell	-7.500	5.145	1.000
		Fort Rucker	-1.250	5.145	1.000
		Patuxent	-12.000	5.145	.207
	Patuxent	Fort Bragg	2.000	5.145	1.000
		Fort Campbell	4.500	5.145	1.000
		Fort Rucker	10.750	5.145	.380
		Ossabaw	12.000	5.145	.207
flag1wid	Fort Bragg	Fort Campbell	-.281	.691	1.000
		Fort Rucker	-1.462	.691	.355
		Ossabaw	-.900	.691	1.000
		Patuxent	-.225	.691	1.000
	Fort Campbell	Fort Bragg	.281	.691	1.000
		Fort Rucker	-1.181	.691	.888
		Ossabaw	-.619	.691	1.000
		Patuxent	.056	.691	1.000
	Fort Rucker	Fort Bragg	1.462	.691	.355
		Fort Campbell	1.181	.691	.888
		Ossabaw	.563	.691	1.000
		Patuxent	1.238	.691	.747
	Ossabaw	Fort Bragg	.900	.691	1.000
		Fort Campbell	.619	.691	1.000
		Fort Rucker	-.563	.691	1.000
		Patuxent	.675	.691	1.000
	Patuxent	Fort Bragg	.225	.691	1.000
		Fort Campbell	-.056	.691	1.000
		Fort Rucker	-1.238	.691	.747
		Ossabaw	-.675	.691	1.000
flag1rat	Fort Bragg	Fort Campbell	.501	.463	1.000
		Fort Rucker	1.416(*)	.463	.025

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Ossabaw	1.152	.463	.137
		Patuxent	.333	.463	1.000
	Fort Campbell	Fort Bragg	-.501	.463	1.000
		Fort Rucker	.915	.463	.495
		Ossabaw	.651	.463	1.000
		Patuxent	-.168	.463	1.000
	Fort Rucker	Fort Bragg	-1.416(*)	.463	.025
		Fort Campbell	-.915	.463	.495
		Ossabaw	-.264	.463	1.000
		Patuxent	-1.084	.463	.203
	Ossabaw	Fort Bragg	-1.152	.463	.137
		Fort Campbell	-.651	.463	1.000
		Fort Rucker	.264	.463	1.000
		Patuxent	-.819	.463	.785
	Patuxent	Fort Bragg	-.333	.463	1.000
		Fort Campbell	.168	.463	1.000
		Fort Rucker	1.084	.463	.203
		Ossabaw	.819	.463	.785
flag2len	Fort Bragg	Fort Campbell	-4.950(*)	1.583	.020
		Fort Rucker	-2.531	1.583	1.000
		Ossabaw	-.219	1.583	1.000
		Patuxent	-5.486(*)	1.604	.008
	Fort Campbell	Fort Bragg	4.950(*)	1.583	.020
		Fort Rucker	2.419	1.583	1.000
		Ossabaw	4.731(*)	1.583	.032
		Patuxent	-.536	1.604	1.000
	Fort Rucker	Fort Bragg	2.531	1.583	1.000
		Fort Campbell	-2.419	1.583	1.000
		Ossabaw	2.313	1.583	1.000
		Patuxent	-2.955	1.604	.670
	Ossabaw	Fort Bragg	.219	1.583	1.000
		Fort Campbell	-4.731(*)	1.583	.032
		Fort Rucker	-2.313	1.583	1.000
		Patuxent	-5.267(*)	1.604	.012
	Patuxent	Fort Bragg	5.486(*)	1.604	.008
		Fort Campbell	.536	1.604	1.000
		Fort Rucker	2.955	1.604	.670
		Ossabaw	5.267(*)	1.604	.012
flag2wid	Fort Bragg	Fort Campbell	.563	.516	1.000
		Fort Rucker	.619	.516	1.000
		Ossabaw	.787	.516	1.000
		Patuxent	.509	.522	1.000
	Fort Campbell	Fort Bragg	-.563	.516	1.000
		Fort Rucker	.056	.516	1.000
		Ossabaw	.225	.516	1.000
		Patuxent	-.053	.522	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Fort Rucker	Fort Bragg	-.619	.516	1.000
		Fort Campbell	-.056	.516	1.000
		Ossabaw	.169	.516	1.000
		Patuxent	-.110	.522	1.000
	Ossabaw	Fort Bragg	-.787	.516	1.000
		Fort Campbell	-.225	.516	1.000
		Fort Rucker	-.169	.516	1.000
		Patuxent	-.278	.522	1.000
	Patuxent	Fort Bragg	-.509	.522	1.000
		Fort Campbell	.053	.522	1.000
		Fort Rucker	.110	.522	1.000
		Ossabaw	.278	.522	1.000
flag2rat	Fort Bragg	Fort Campbell	-.406	.194	.375
		Fort Rucker	-.307	.194	1.000
		Ossabaw	-.270	.194	1.000
		Patuxent	-.473	.197	.170
	Fort Campbell	Fort Bragg	.406	.194	.375
		Fort Rucker	.099	.194	1.000
		Ossabaw	.136	.194	1.000
		Patuxent	-.067	.197	1.000
	Fort Rucker	Fort Bragg	.307	.194	1.000
		Fort Campbell	-.099	.194	1.000
		Ossabaw	.037	.194	1.000
		Patuxent	-.166	.197	1.000
	Ossabaw	Fort Bragg	.270	.194	1.000
		Fort Campbell	-.136	.194	1.000
		Fort Rucker	-.037	.194	1.000
		Patuxent	-.203	.197	1.000
	Patuxent	Fort Bragg	.473	.197	.170
		Fort Campbell	.067	.197	1.000
		Fort Rucker	.166	.197	1.000
		Ossabaw	.203	.197	1.000
flag3len	Fort Bragg	Fort Campbell	-1.744	1.745	1.000
		Fort Rucker	-.225	1.745	1.000
		Ossabaw	1.912	1.745	1.000
		Patuxent	-2.171	1.780	1.000
	Fort Campbell	Fort Bragg	1.744	1.745	1.000
		Fort Rucker	1.519	1.745	1.000
		Ossabaw	3.656	1.745	.374
		Patuxent	-.427	1.780	1.000
	Fort Rucker	Fort Bragg	.225	1.745	1.000
		Fort Campbell	-1.519	1.745	1.000
		Ossabaw	2.137	1.745	1.000
		Patuxent	-1.946	1.780	1.000
	Ossabaw	Fort Bragg	-1.912	1.745	1.000
		Fort Campbell	-3.656	1.745	.374

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Rucker	-2.137	1.745	1.000
		Patuxent	-4.083	1.780	.228
	Patuxent	Fort Bragg	2.171	1.780	1.000
		Fort Campbell	.427	1.780	1.000
		Fort Rucker	1.946	1.780	1.000
		Ossabaw	4.083	1.780	.228
flag3wid	Fort Bragg	Fort Campbell	.900	.561	1.000
		Fort Rucker	.956	.561	.896
		Ossabaw	1.125	.561	.462
		Patuxent	.382	.572	1.000
	Fort Campbell	Fort Bragg	-.900	.561	1.000
		Fort Rucker	.056	.561	1.000
		Ossabaw	.225	.561	1.000
		Patuxent	-.518	.572	1.000
	Fort Rucker	Fort Bragg	-.956	.561	.896
		Fort Campbell	-.056	.561	1.000
		Ossabaw	.169	.561	1.000
		Patuxent	-.575	.572	1.000
	Ossabaw	Fort Bragg	-1.125	.561	.462
		Fort Campbell	-.225	.561	1.000
		Fort Rucker	-.169	.561	1.000
		Patuxent	-.743	.572	1.000
	Patuxent	Fort Bragg	-.382	.572	1.000
		Fort Campbell	.518	.572	1.000
		Fort Rucker	.575	.572	1.000
		Ossabaw	.743	.572	1.000
flag3rat	Fort Bragg	Fort Campbell	-.456	.190	.174
		Fort Rucker	-.338	.190	.768
		Ossabaw	-.107	.191	1.000
		Patuxent	-.260	.194	1.000
	Fort Campbell	Fort Bragg	.456	.190	.174
		Fort Rucker	.118	.190	1.000
		Ossabaw	.349	.191	.698
		Patuxent	.196	.194	1.000
	Fort Rucker	Fort Bragg	.338	.190	.768
		Fort Campbell	-.118	.190	1.000
		Ossabaw	.231	.191	1.000
		Patuxent	.078	.194	1.000
	Ossabaw	Fort Bragg	.107	.191	1.000
		Fort Campbell	-.349	.191	.698
		Fort Rucker	-.231	.191	1.000
		Patuxent	-.153	.195	1.000
	Patuxent	Fort Bragg	.260	.194	1.000
		Fort Campbell	-.196	.194	1.000
		Fort Rucker	-.078	.194	1.000
		Ossabaw	.153	.195	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
flag4len	Fort Bragg	Fort Campbell	-2.981	1.493	.472
		Fort Rucker	-.225	1.493	1.000
		Ossabaw	1.688	1.493	1.000
		Patuxent	-.544	1.534	1.000
	Fort Campbell	Fort Bragg	2.981	1.493	.472
		Fort Rucker	2.756	1.493	.664
		Ossabaw	4.669(*)	1.493	.020
		Patuxent	2.438	1.534	1.000
	Fort Rucker	Fort Bragg	.225	1.493	1.000
		Fort Campbell	-2.756	1.493	.664
		Ossabaw	1.912	1.493	1.000
		Patuxent	-.319	1.534	1.000
	Ossabaw	Fort Bragg	-1.688	1.493	1.000
		Fort Campbell	-4.669(*)	1.493	.020
		Fort Rucker	-1.912	1.493	1.000
		Patuxent	-2.231	1.534	1.000
	Patuxent	Fort Bragg	.544	1.534	1.000
		Fort Campbell	-2.438	1.534	1.000
		Fort Rucker	.319	1.534	1.000
		Ossabaw	2.231	1.534	1.000
flag4wid	Fort Bragg	Fort Campbell	1.406(*)	.470	.031
		Fort Rucker	1.519(*)	.470	.015
		Ossabaw	1.069	.473	.250
		Patuxent	.812	.487	.970
	Fort Campbell	Fort Bragg	-1.406(*)	.470	.031
		Fort Rucker	.113	.470	1.000
		Ossabaw	-.337	.473	1.000
		Patuxent	-.595	.487	1.000
	Fort Rucker	Fort Bragg	-1.519(*)	.470	.015
		Fort Campbell	-.113	.470	1.000
		Ossabaw	-.450	.473	1.000
		Patuxent	-.707	.487	1.000
	Ossabaw	Fort Bragg	-1.069	.473	.250
		Fort Campbell	.337	.473	1.000
		Fort Rucker	.450	.473	1.000
		Patuxent	-.257	.490	1.000
	Patuxent	Fort Bragg	-.812	.487	.970
		Fort Campbell	.595	.487	1.000
		Fort Rucker	.707	.487	1.000
		Ossabaw	.257	.490	1.000
flag4rat	Fort Bragg	Fort Campbell	-.652(*)	.187	.006
		Fort Rucker	-.532(*)	.187	.048
		Ossabaw	-.327	.188	.833
		Patuxent	-.332	.193	.871
	Fort Campbell	Fort Bragg	.652(*)	.187	.006
		Fort Rucker	.120	.187	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Ossabaw	.325	.188	.848
		Patuxent	.320	.193	.992
	Fort Rucker	Fort Bragg	.532(*)	.187	.048
		Fort Campbell	-.120	.187	1.000
		Ossabaw	.205	.188	1.000
		Patuxent	.200	.193	1.000
	Ossabaw	Fort Bragg	.327	.188	.833
		Fort Campbell	-.325	.188	.848
		Fort Rucker	-.205	.188	1.000
		Patuxent	-.005	.194	1.000
	Patuxent	Fort Bragg	.332	.193	.871
		Fort Campbell	-.320	.193	.992
		Fort Rucker	-.200	.193	1.000
		Ossabaw	.005	.194	1.000
flag5len	Fort Bragg	Fort Campbell	-3.769	1.460	.106
		Fort Rucker	-.731	1.460	1.000
		Ossabaw	1.181	1.460	1.000
		Patuxent	-1.756	1.500	1.000
	Fort Campbell	Fort Bragg	3.769	1.460	.106
		Fort Rucker	3.037	1.460	.388
		Ossabaw	4.950(*)	1.460	.008
		Patuxent	2.012	1.500	1.000
	Fort Rucker	Fort Bragg	.731	1.460	1.000
		Fort Campbell	-3.037	1.460	.388
		Ossabaw	1.912	1.460	1.000
		Patuxent	-1.025	1.500	1.000
	Ossabaw	Fort Bragg	-1.181	1.460	1.000
		Fort Campbell	-4.950(*)	1.460	.008
		Fort Rucker	-1.912	1.460	1.000
		Patuxent	-2.938	1.500	.517
	Patuxent	Fort Bragg	1.756	1.500	1.000
		Fort Campbell	-2.012	1.500	1.000
		Fort Rucker	1.025	1.500	1.000
		Ossabaw	2.938	1.500	.517
flag5wid	Fort Bragg	Fort Campbell	1.688(*)	.435	.001
		Fort Rucker	1.462(*)	.435	.009
		Ossabaw	1.969(*)	.435	.000
		Patuxent	1.063	.447	.185
	Fort Campbell	Fort Bragg	-1.688(*)	.435	.001
		Fort Rucker	-.225	.435	1.000
		Ossabaw	.281	.435	1.000
		Patuxent	-.625	.447	1.000
	Fort Rucker	Fort Bragg	-1.462(*)	.435	.009
		Fort Campbell	.225	.435	1.000
		Ossabaw	.506	.435	1.000
		Patuxent	-.400	.447	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Ossabaw	Fort Bragg	-1.969(*)	.435	.000
		Fort Campbell	-.281	.435	1.000
		Fort Rucker	-.506	.435	1.000
		Patuxent	-.906	.447	.441
	Patuxent	Fort Bragg	-1.063	.447	.185
		Fort Campbell	.625	.447	1.000
		Fort Rucker	.400	.447	1.000
		Ossabaw	.906	.447	.441
flag5rat	Fort Bragg	Fort Campbell	-.820(*)	.178	7.095E-05
		Fort Rucker	-.538(*)	.178	.028
		Ossabaw	-.638(*)	.178	.004
		Patuxent	-.474	.183	.102
	Fort Campbell	Fort Bragg	.820(*)	.178	7.095E-05
		Fort Rucker	.282	.178	1.000
		Ossabaw	.182	.178	1.000
		Patuxent	.347	.183	.591
	Fort Rucker	Fort Bragg	.538(*)	.178	.028
		Fort Campbell	-.282	.178	1.000
		Ossabaw	-.100	.178	1.000
		Patuxent	.064	.183	1.000
	Ossabaw	Fort Bragg	.638(*)	.178	.004
		Fort Campbell	-.182	.178	1.000
		Fort Rucker	.100	.178	1.000
		Patuxent	.165	.183	1.000
	Patuxent	Fort Bragg	.474	.183	.102
		Fort Campbell	-.347	.183	.591
		Fort Rucker	-.064	.183	1.000
		Ossabaw	-.165	.183	1.000
flag6len	Fort Bragg	Fort Campbell	-3.487	1.456	.176
		Fort Rucker	-.619	1.456	1.000
		Ossabaw	1.398	1.465	1.000
		Patuxent	-2.306	1.507	1.000
	Fort Campbell	Fort Bragg	3.487	1.456	.176
		Fort Rucker	2.869	1.456	.502
		Ossabaw	4.885(*)	1.465	.010
		Patuxent	1.181	1.507	1.000
	Fort Rucker	Fort Bragg	.619	1.456	1.000
		Fort Campbell	-2.869	1.456	.502
		Ossabaw	2.016	1.465	1.000
		Patuxent	-1.688	1.507	1.000
	Ossabaw	Fort Bragg	-1.398	1.465	1.000
		Fort Campbell	-4.885(*)	1.465	.010
		Fort Rucker	-2.016	1.465	1.000
		Patuxent	-3.704	1.516	.155
	Patuxent	Fort Bragg	2.306	1.507	1.000
		Fort Campbell	-1.181	1.507	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Rucker	1.688	1.507	1.000
		Ossabaw	3.704	1.516	.155
flag6wid	Fort Bragg	Fort Campbell	.632	.461	1.000
		Fort Rucker	1.631(*)	.458	.005
		Ossabaw	2.016(*)	.461	.000
		Patuxent	.708	.478	1.000
	Fort Campbell	Fort Bragg	-.632	.461	1.000
		Fort Rucker	1.000	.461	.316
		Ossabaw	1.385(*)	.464	.032
		Patuxent	.076	.481	1.000
	Fort Rucker	Fort Bragg	-1.631(*)	.458	.005
		Fort Campbell	-1.000	.461	.316
		Ossabaw	.385	.461	1.000
		Patuxent	-.923	.478	.551
	Ossabaw	Fort Bragg	-2.016(*)	.461	.000
		Fort Campbell	-1.385(*)	.464	.032
		Fort Rucker	-.385	.461	1.000
		Patuxent	-1.308	.481	.072
	Patuxent	Fort Bragg	-.708	.478	1.000
		Fort Campbell	-.076	.481	1.000
		Fort Rucker	.923	.478	.551
		Ossabaw	1.308	.481	.072
flag6rat	Fort Bragg	Fort Campbell	-.302	.199	1.000
		Fort Rucker	-.562(*)	.198	.049
		Ossabaw	-.609(*)	.199	.025
		Patuxent	-.311	.206	1.000
	Fort Campbell	Fort Bragg	.302	.199	1.000
		Fort Rucker	-.260	.199	1.000
		Ossabaw	-.307	.200	1.000
		Patuxent	-.009	.207	1.000
	Fort Rucker	Fort Bragg	.562(*)	.198	.049
		Fort Campbell	.260	.199	1.000
		Ossabaw	-.047	.199	1.000
		Patuxent	.251	.206	1.000
	Ossabaw	Fort Bragg	.609(*)	.199	.025
		Fort Campbell	.307	.200	1.000
		Fort Rucker	.047	.199	1.000
		Patuxent	.298	.207	1.000
	Patuxent	Fort Bragg	.311	.206	1.000
		Fort Campbell	.009	.207	1.000
		Fort Rucker	-.251	.206	1.000
		Ossabaw	-.298	.207	1.000
flag7len	Fort Bragg	Fort Campbell	-3.865	1.616	.178
		Fort Rucker	-2.654	1.616	1.000
		Ossabaw	-.937	1.638	1.000
		Patuxent	-4.311	1.688	.115

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Fort Campbell	Fort Bragg	3.865	1.616	.178
		Fort Rucker	1.212	1.616	1.000
		Ossabaw	2.928	1.638	.754
		Patuxent	-.446	1.688	1.000
	Fort Rucker	Fort Bragg	2.654	1.616	1.000
		Fort Campbell	-1.212	1.616	1.000
		Ossabaw	1.717	1.638	1.000
		Patuxent	-1.657	1.688	1.000
	Ossabaw	Fort Bragg	.937	1.638	1.000
		Fort Campbell	-2.928	1.638	.754
		Fort Rucker	-1.717	1.638	1.000
		Patuxent	-3.374	1.709	.498
	Patuxent	Fort Bragg	4.311	1.688	.115
		Fort Campbell	.446	1.688	1.000
		Fort Rucker	1.657	1.688	1.000
		Ossabaw	3.374	1.709	.498
flag7wid	Fort Bragg	Fort Campbell	.462	.462	1.000
		Fort Rucker	1.788(*)	.462	.002
		Ossabaw	1.439(*)	.468	.024
		Patuxent	.614	.482	1.000
	Fort Campbell	Fort Bragg	-.462	.462	1.000
		Fort Rucker	1.327(*)	.462	.046
		Ossabaw	.978	.468	.381
		Patuxent	.152	.482	1.000
	Fort Rucker	Fort Bragg	-1.788(*)	.462	.002
		Fort Campbell	-1.327(*)	.462	.046
		Ossabaw	-.349	.468	1.000
		Patuxent	-1.175	.482	.158
	Ossabaw	Fort Bragg	-1.439(*)	.468	.024
		Fort Campbell	-.978	.468	.381
		Fort Rucker	.349	.468	1.000
		Patuxent	-.826	.488	.927
	Patuxent	Fort Bragg	-.614	.482	1.000
		Fort Campbell	-.152	.482	1.000
		Fort Rucker	1.175	.482	.158
		Ossabaw	.826	.488	.927
flag7rat	Fort Bragg	Fort Campbell	-.360	.194	.652
		Fort Rucker	-.827(*)	.194	.000
		Ossabaw	-.569(*)	.197	.042
		Patuxent	-.475	.203	.202
	Fort Campbell	Fort Bragg	.360	.194	.652
		Fort Rucker	-.468	.194	.169
		Ossabaw	-.209	.197	1.000
		Patuxent	-.115	.203	1.000
	Fort Rucker	Fort Bragg	.827(*)	.194	.000
		Fort Campbell	.468	.194	.169

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Ossabaw	.258	.197	1.000
		Patuxent	.353	.203	.833
	Ossabaw	Fort Bragg	.569(*)	.197	.042
		Fort Campbell	.209	.197	1.000
		Fort Rucker	-.258	.197	1.000
		Patuxent	.094	.205	1.000
	Patuxent	Fort Bragg	.475	.203	.202
		Fort Campbell	.115	.203	1.000
		Fort Rucker	-.353	.203	.833
		Ossabaw	-.094	.205	1.000
r5l	Fort Bragg	Fort Campbell	-9.942	12.446	1.000
		Fort Rucker	-1.142	12.446	1.000
		Ossabaw	24.470	12.692	.554
		Patuxent	-49.359(*)	12.783	.002
	Fort Campbell	Fort Bragg	9.942	12.446	1.000
		Fort Rucker	8.800	12.367	1.000
		Ossabaw	34.412	12.615	.070
		Patuxent	-39.417(*)	12.706	.022
	Fort Rucker	Fort Bragg	1.142	12.446	1.000
		Fort Campbell	-8.800	12.367	1.000
		Ossabaw	25.612	12.615	.437
		Patuxent	-48.217(*)	12.706	.002
	Ossabaw	Fort Bragg	-24.470	12.692	.554
		Fort Campbell	-34.412	12.615	.070
		Fort Rucker	-25.612	12.615	.437
		Patuxent	-73.829(*)	12.947	4.550E-07
	Patuxent	Fort Bragg	49.359(*)	12.783	.002
		Fort Campbell	39.417(*)	12.706	.022
		Fort Rucker	48.217(*)	12.706	.002
		Ossabaw	73.829(*)	12.947	4.550E-07
wingwid	Fort Bragg	Fort Campbell	-.810	6.954	1.000
		Fort Rucker	-5.079	6.911	1.000
		Ossabaw	8.421	7.046	1.000
		Patuxent	-34.822(*)	7.046	1.717E-05
	Fort Campbell	Fort Bragg	.810	6.954	1.000
		Fort Rucker	-4.269	6.865	1.000
		Ossabaw	9.231	7.001	1.000
		Patuxent	-34.012(*)	7.001	2.508E-05
	Fort Rucker	Fort Bragg	5.079	6.911	1.000
		Fort Campbell	4.269	6.865	1.000
		Ossabaw	13.500	6.958	.539
		Patuxent	-29.743(*)	6.958	.000
	Ossabaw	Fort Bragg	-8.421	7.046	1.000
		Fort Campbell	-9.231	7.001	1.000
		Fort Rucker	-13.500	6.958	.539
		Patuxent	-43.243(*)	7.093	6.111E-08

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Patuxent	Fort Bragg	34.822(*)	7.046	1.717E-05
		Fort Campbell	34.012(*)	7.001	2.508E-05
		Fort Rucker	29.743(*)	6.958	.000
		Ossabaw	43.243(*)	7.093	6.111E-08
alpha	Fort Bragg	Fort Campbell	-22.584	10.728	.366
		Fort Rucker	-3.545	10.591	1.000
		Ossabaw	15.837	10.728	1.000
		Patuxent	-42.065(*)	10.801	.001
	Fort Campbell	Fort Bragg	22.584	10.728	.366
		Fort Rucker	19.039	10.661	.757
		Ossabaw	38.421(*)	10.797	.005
		Patuxent	-19.481	10.870	.747
	Fort Rucker	Fort Bragg	3.545	10.591	1.000
		Fort Campbell	-19.039	10.661	.757
		Ossabaw	19.382	10.661	.707
		Patuxent	-38.520(*)	10.735	.004
	Ossabaw	Fort Bragg	-15.837	10.728	1.000
		Fort Campbell	-38.421(*)	10.797	.005
		Fort Rucker	-19.382	10.661	.707
		Patuxent	-57.902(*)	10.870	2.852E-06
	Patuxent	Fort Bragg	42.065(*)	10.801	.001
		Fort Campbell	19.481	10.870	.747
		Fort Rucker	38.520(*)	10.735	.004
		Ossabaw	57.902(*)	10.870	2.852E-06
delta	Fort Bragg	Fort Campbell	-23.846	8.908	.081
		Fort Rucker	-20.205	8.852	.236
		Ossabaw	-3.205	8.967	1.000
		Patuxent	-27.665(*)	9.028	.025
	Fort Campbell	Fort Bragg	23.846	8.908	.081
		Fort Rucker	3.641	8.852	1.000
		Ossabaw	20.641	8.967	.224
		Patuxent	-3.818	9.028	1.000
	Fort Rucker	Fort Bragg	20.205	8.852	.236
		Fort Campbell	-3.641	8.852	1.000
		Ossabaw	17.000	8.911	.579
		Patuxent	-7.459	8.973	1.000
	Ossabaw	Fort Bragg	3.205	8.967	1.000
		Fort Campbell	-20.641	8.967	.224
		Fort Rucker	-17.000	8.911	.579
		Patuxent	-24.459	9.085	.077
	Patuxent	Fort Bragg	27.665(*)	9.028	.025
		Fort Campbell	3.818	9.028	1.000
		Fort Rucker	7.459	8.973	1.000
		Ossabaw	24.459	9.085	.077
beta	Fort Bragg	Fort Campbell	11.026	7.171	1.000
		Fort Rucker	8.859	7.126	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Ossabaw	6.410	7.171	1.000
		Patuxent	-4.830	7.267	1.000
	Fort Campbell	Fort Bragg	-11.026	7.171	1.000
		Fort Rucker	-2.167	7.126	1.000
		Ossabaw	-4.615	7.171	1.000
		Patuxent	-15.856	7.267	.304
	Fort Rucker	Fort Bragg	-8.859	7.126	1.000
		Fort Campbell	2.167	7.126	1.000
		Ossabaw	-2.449	7.126	1.000
		Patuxent	-13.689	7.223	.596
	Ossabaw	Fort Bragg	-6.410	7.171	1.000
		Fort Campbell	4.615	7.171	1.000
		Fort Rucker	2.449	7.126	1.000
		Patuxent	-11.240	7.267	1.000
	Patuxent	Fort Bragg	4.830	7.267	1.000
		Fort Campbell	15.856	7.267	.304
		Fort Rucker	13.689	7.223	.596
		Ossabaw	11.240	7.267	1.000
gamma	Fort Bragg	Fort Campbell	16.923	6.503	.100
		Fort Rucker	9.340	6.462	1.000
		Ossabaw	18.462	6.503	.050
		Patuxent	10.887	6.590	1.000
	Fort Campbell	Fort Bragg	-16.923	6.503	.100
		Fort Rucker	-7.583	6.462	1.000
		Ossabaw	1.538	6.503	1.000
		Patuxent	-6.036	6.590	1.000
	Fort Rucker	Fort Bragg	-9.340	6.462	1.000
		Fort Campbell	7.583	6.462	1.000
		Ossabaw	9.122	6.462	1.000
		Patuxent	1.547	6.550	1.000
	Ossabaw	Fort Bragg	-18.462	6.503	.050
		Fort Campbell	-1.538	6.503	1.000
		Fort Rucker	-9.122	6.462	1.000
		Patuxent	-7.574	6.590	1.000
	Patuxent	Fort Bragg	-10.887	6.590	1.000
		Fort Campbell	6.036	6.590	1.000
		Fort Rucker	-1.547	6.550	1.000
		Ossabaw	7.574	6.590	1.000
femur	Fort Bragg	Fort Campbell	-4.245	13.398	1.000
		Fort Rucker	1.772	13.677	1.000
		Ossabaw	57.436(*)	13.780	.000
		Patuxent	45.493(*)	13.780	.012
	Fort Campbell	Fort Bragg	4.245	13.398	1.000
		Fort Rucker	6.017	13.760	1.000
		Ossabaw	61.681(*)	13.862	.000
		Patuxent	49.738(*)	13.862	.004

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Fort Rucker	Fort Bragg	-1.772	13.677	1.000
		Fort Campbell	-6.017	13.760	1.000
		Ossabaw	55.663(*)	14.133	.001
		Patuxent	43.721(*)	14.133	.023
	Ossabaw	Fort Bragg	-57.436(*)	13.780	.000
		Fort Campbell	-61.681(*)	13.862	.000
		Fort Rucker	-55.663(*)	14.133	.001
		Patuxent	-11.943	14.232	1.000
	Patuxent	Fort Bragg	-45.493(*)	13.780	.012
		Fort Campbell	-49.738(*)	13.862	.004
		Fort Rucker	-43.721(*)	14.133	.023
		Ossabaw	11.943	14.232	1.000
tibia	Fort Bragg	Fort Campbell	1.326	23.941	1.000
		Fort Rucker	10.126	23.941	1.000
		Ossabaw	131.512(*)	24.416	2.143E-06
		Patuxent	28.581	24.589	1.000
	Fort Campbell	Fort Bragg	-1.326	23.941	1.000
		Fort Rucker	8.800	23.789	1.000
		Ossabaw	130.186(*)	24.267	2.376E-06
		Patuxent	27.256	24.441	1.000
	Fort Rucker	Fort Bragg	-10.126	23.941	1.000
		Fort Campbell	-8.800	23.789	1.000
		Ossabaw	121.386(*)	24.267	1.301E-05
		Patuxent	18.456	24.441	1.000
	Ossabaw	Fort Bragg	-131.512(*)	24.416	2.143E-06
		Fort Campbell	-130.186(*)	24.267	2.376E-06
		Fort Rucker	-121.386(*)	24.267	1.301E-05
		Patuxent	-102.931(*)	24.906	.001
	Patuxent	Fort Bragg	-28.581	24.589	1.000
		Fort Campbell	-27.256	24.441	1.000
		Fort Rucker	-18.456	24.441	1.000
		Ossabaw	102.931(*)	24.906	.001
gstyllen	Fort Bragg	Fort Campbell	3.656	2.004	.697
		Fort Rucker	-.787	2.004	1.000
		Ossabaw	3.938	2.004	.509
		Patuxent	1.744	2.004	1.000
	Fort Campbell	Fort Bragg	-3.656	2.004	.697
		Fort Rucker	-4.444	2.004	.278
		Ossabaw	.281	2.004	1.000
		Patuxent	-1.912	2.004	1.000
	Fort Rucker	Fort Bragg	.787	2.004	1.000
		Fort Campbell	4.444	2.004	.278
		Ossabaw	4.725	2.004	.194
		Patuxent	2.531	2.004	1.000
	Ossabaw	Fort Bragg	-3.938	2.004	.509
		Fort Campbell	-.281	2.004	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Rucker	-4.725	2.004	.194
		Patuxent	-2.194	2.004	1.000
	Patuxent	Fort Bragg	-1.744	2.004	1.000
		Fort Campbell	1.912	2.004	1.000
		Fort Rucker	-2.531	2.004	1.000
		Ossabaw	2.194	2.004	1.000
gstylwid	Fort Bragg	Fort Campbell	2.869	1.443	.482
		Fort Rucker	.844	1.443	1.000
		Ossabaw	-.956	1.443	1.000
		Patuxent	-4.444(*)	1.443	.024
	Fort Campbell	Fort Bragg	-2.869	1.443	.482
		Fort Rucker	-2.025	1.443	1.000
		Ossabaw	-3.825	1.443	.087
		Patuxent	-7.313(*)	1.443	9.347E-06
	Fort Rucker	Fort Bragg	-.844	1.443	1.000
		Fort Campbell	2.025	1.443	1.000
		Ossabaw	-1.800	1.443	1.000
		Patuxent	-5.288(*)	1.443	.003
	Ossabaw	Fort Bragg	.956	1.443	1.000
		Fort Campbell	3.825	1.443	.087
		Fort Rucker	1.800	1.443	1.000
		Patuxent	-3.488	1.443	.166
	Patuxent	Fort Bragg	4.444(*)	1.443	.024
		Fort Campbell	7.313(*)	1.443	9.347E-06
		Fort Rucker	5.288(*)	1.443	.003
		Ossabaw	3.488	1.443	.166
gstylrat	Fort Bragg	Fort Campbell	-.202	.171	1.000
		Fort Rucker	-.027	.171	1.000
		Ossabaw	.261	.171	1.000
		Patuxent	.628(*)	.171	.003
	Fort Campbell	Fort Bragg	.202	.171	1.000
		Fort Rucker	.175	.171	1.000
		Ossabaw	.463	.171	.073
		Patuxent	.830(*)	.171	2.375E-05
	Fort Rucker	Fort Bragg	.027	.171	1.000
		Fort Campbell	-.175	.171	1.000
		Ossabaw	.288	.171	.931
		Patuxent	.655(*)	.171	.002
	Ossabaw	Fort Bragg	-.261	.171	1.000
		Fort Campbell	-.463	.171	.073
		Fort Rucker	-.288	.171	.931
		Patuxent	.367	.171	.328
	Patuxent	Fort Bragg	-.628(*)	.171	.003
		Fort Campbell	-.830(*)	.171	2.375E-05
		Fort Rucker	-.655(*)	.171	.002
		Ossabaw	-.367	.171	.328

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
gcxlen	Fort Bragg	Fort Campbell	5.000	4.995	1.000
		Fort Rucker	6.250	4.995	1.000
		Ossabaw	19.000(*)	4.995	.002
		Patuxent	1.000	4.995	1.000
	Fort Campbell	Fort Bragg	-5.000	4.995	1.000
		Fort Rucker	1.250	4.995	1.000
		Ossabaw	14.000	4.995	.056
		Patuxent	-4.000	4.995	1.000
	Fort Rucker	Fort Bragg	-6.250	4.995	1.000
		Fort Campbell	-1.250	4.995	1.000
		Ossabaw	12.750	4.995	.115
		Patuxent	-5.250	4.995	1.000
	Ossabaw	Fort Bragg	-19.000(*)	4.995	.002
		Fort Campbell	-14.000	4.995	.056
		Fort Rucker	-12.750	4.995	.115
		Patuxent	-18.000(*)	4.995	.004
	Patuxent	Fort Bragg	-1.000	4.995	1.000
		Fort Campbell	4.000	4.995	1.000
		Fort Rucker	5.250	4.995	1.000
		Ossabaw	18.000(*)	4.995	.004
gcxwid	Fort Bragg	Fort Campbell	-.250	1.741	1.000
		Fort Rucker	-1.500	1.741	1.000
		Ossabaw	.000	1.741	1.000
		Patuxent	-4.250	1.741	.156
	Fort Campbell	Fort Bragg	.250	1.741	1.000
		Fort Rucker	-1.250	1.741	1.000
		Ossabaw	.250	1.741	1.000
		Patuxent	-4.000	1.741	.227
	Fort Rucker	Fort Bragg	1.500	1.741	1.000
		Fort Campbell	1.250	1.741	1.000
		Ossabaw	1.500	1.741	1.000
		Patuxent	-2.750	1.741	1.000
	Ossabaw	Fort Bragg	.000	1.741	1.000
		Fort Campbell	-.250	1.741	1.000
		Fort Rucker	-1.500	1.741	1.000
		Patuxent	-4.250	1.741	.156
	Patuxent	Fort Bragg	4.250	1.741	.156
		Fort Campbell	4.000	1.741	.227
		Fort Rucker	2.750	1.741	1.000
		Ossabaw	4.250	1.741	.156
gcxrat	Fort Bragg	Fort Campbell	.081	.084	1.000
		Fort Rucker	.135	.084	1.000
		Ossabaw	.228	.084	.070
		Patuxent	.211	.084	.125
	Fort Campbell	Fort Bragg	-.081	.084	1.000
		Fort Rucker	.054	.084	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Ossabaw	.148	.084	.794
		Patuxent	.131	.084	1.000
	Fort Rucker	Fort Bragg	-.135	.084	1.000
		Fort Campbell	-.054	.084	1.000
		Ossabaw	.094	.084	1.000
		Patuxent	.077	.084	1.000
	Ossabaw	Fort Bragg	-.228	.084	.070
		Fort Campbell	-.148	.084	.794
		Fort Rucker	-.094	.084	1.000
		Patuxent	-.017	.084	1.000
	Patuxent	Fort Bragg	-.211	.084	.125
		Fort Campbell	-.131	.084	1.000
		Fort Rucker	-.077	.084	1.000
		Ossabaw	.017	.084	1.000
latlbl	Fort Bragg	Fort Campbell	-3.000	4.812	1.000
		Fort Rucker	-3.750	4.812	1.000
		Ossabaw	-1.500	4.812	1.000
		Patuxent	3.750	4.812	1.000
	Fort Campbell	Fort Bragg	3.000	4.812	1.000
		Fort Rucker	-.750	4.812	1.000
		Ossabaw	1.500	4.812	1.000
		Patuxent	6.750	4.812	1.000
	Fort Rucker	Fort Bragg	3.750	4.812	1.000
		Fort Campbell	.750	4.812	1.000
		Ossabaw	2.250	4.812	1.000
		Patuxent	7.500	4.812	1.000
	Ossabaw	Fort Bragg	1.500	4.812	1.000
		Fort Campbell	-1.500	4.812	1.000
		Fort Rucker	-2.250	4.812	1.000
		Patuxent	5.250	4.812	1.000
	Patuxent	Fort Bragg	-3.750	4.812	1.000
		Fort Campbell	-6.750	4.812	1.000
		Fort Rucker	-7.500	4.812	1.000
		Ossabaw	-5.250	4.812	1.000
latlbw	Fort Bragg	Fort Campbell	-.788	1.119	1.000
		Fort Rucker	1.069	1.119	1.000
		Ossabaw	-.394	1.119	1.000
		Patuxent	-2.588	1.119	.218
	Fort Campbell	Fort Bragg	.788	1.119	1.000
		Fort Rucker	1.856	1.119	.988
		Ossabaw	.394	1.119	1.000
		Patuxent	-1.800	1.119	1.000
	Fort Rucker	Fort Bragg	-1.069	1.119	1.000
		Fort Campbell	-1.856	1.119	.988
		Ossabaw	-1.462	1.119	1.000
		Patuxent	-3.656(*)	1.119	.013

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Ossabaw	Fort Bragg	.394	1.119	1.000
		Fort Campbell	-.394	1.119	1.000
		Fort Rucker	1.462	1.119	1.000
		Patuxent	-2.194	1.119	.514
	Patuxent	Fort Bragg	2.588	1.119	.218
		Fort Campbell	1.800	1.119	1.000
		Fort Rucker	3.656(*)	1.119	.013
		Ossabaw	2.194	1.119	.514
latlbrat	Fort Bragg	Fort Campbell	.086	.337	1.000
		Fort Rucker	-.362	.337	1.000
		Ossabaw	.052	.337	1.000
		Patuxent	.845	.337	.130
	Fort Campbell	Fort Bragg	-.086	.337	1.000
		Fort Rucker	-.448	.337	1.000
		Ossabaw	-.034	.337	1.000
		Patuxent	.758	.337	.256
	Fort Rucker	Fort Bragg	.362	.337	1.000
		Fort Campbell	.448	.337	1.000
		Ossabaw	.414	.337	1.000
		Patuxent	1.207(*)	.337	.004
	Ossabaw	Fort Bragg	-.052	.337	1.000
		Fort Campbell	.034	.337	1.000
		Fort Rucker	-.414	.337	1.000
		Patuxent	.793	.337	.197
	Patuxent	Fort Bragg	-.845	.337	.130
		Fort Campbell	-.758	.337	.256
		Fort Rucker	-1.207(*)	.337	.004
		Ossabaw	-.793	.337	.197

* The mean difference is significant at the .05 level.

Appendix D

Multiple comparisons of female data

Appendix D: Multiple comparisons of female data
Mean Difference in microns
*** The mean difference is significant at the .05 level**

Bonferroni

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
hdlength	Fort Bragg	Fort Campbell	18.060(*)	5.570	.020
		Fort Rucker	-2.821	5.497	1.000
		Suwannee	11.687	5.533	.536
		Ossabaw	19.055(*)	5.533	.010
		Patuxent	10.897	5.462	.709
	Fort Campbell	Fort Bragg	-18.060(*)	5.570	.020
		Fort Rucker	-20.880(*)	5.570	.003
		Suwannee	-6.373	5.606	1.000
		Ossabaw	.996	5.606	1.000
		Patuxent	-7.162	5.536	1.000
	Fort Rucker	Fort Bragg	2.821	5.497	1.000
		Fort Campbell	20.880(*)	5.570	.003
		Suwannee	14.507	5.533	.140
		Ossabaw	21.876(*)	5.533	.002
		Patuxent	13.718	5.462	.191
	Suwannee	Fort Bragg	-11.687	5.533	.536
		Fort Campbell	6.373	5.606	1.000
		Fort Rucker	-14.507	5.533	.140
		Ossabaw	7.368	5.568	1.000
		Patuxent	-.789	5.498	1.000
	Ossabaw	Fort Bragg	-19.055(*)	5.533	.010
		Fort Campbell	-.996	5.606	1.000
		Fort Rucker	-21.876(*)	5.533	.002
		Suwannee	-7.368	5.568	1.000
		Patuxent	-8.158	5.498	1.000
	Patuxent	Fort Bragg	-10.897	5.462	.709
		Fort Campbell	7.162	5.536	1.000
		Fort Rucker	-13.718	5.462	.191
		Suwannee	.789	5.498	1.000
		Ossabaw	8.158	5.498	1.000
clypeus	Fort Bragg	Fort Campbell	4.331	2.229	.797
		Fort Rucker	-1.969	2.229	1.000
		Suwannee	5.513	2.229	.211
		Ossabaw	1.527	2.243	1.000
		Patuxent	-6.019	2.229	.111
	Fort Campbell	Fort Bragg	-4.331	2.229	.797
		Fort Rucker	-6.300	2.229	.077
		Suwannee	1.181	2.229	1.000
		Ossabaw	-2.804	2.243	1.000
		Patuxent	-10.350(*)	2.229	8.555E-05
	Fort Rucker	Fort Bragg	1.969	2.229	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	6.300	2.229	.077
		Suwannee	7.481(*)	2.229	.014
		Ossabaw	3.496	2.243	1.000
		Patuxent	-4.050	2.229	1.000
	Suwannee	Fort Bragg	-5.513	2.229	.211
		Fort Campbell	-1.181	2.229	1.000
		Fort Rucker	-7.481(*)	2.229	.014
		Ossabaw	-3.985	2.243	1.000
		Patuxent	-11.531(*)	2.229	7.396E-06
	Ossabaw	Fort Bragg	-1.527	2.243	1.000
		Fort Campbell	2.804	2.243	1.000
		Fort Rucker	-3.496	2.243	1.000
		Suwannee	3.985	2.243	1.000
		Patuxent	-7.546(*)	2.243	.013
	Patuxent	Fort Bragg	6.019	2.229	.111
		Fort Campbell	10.350(*)	2.229	8.555E-05
		Fort Rucker	4.050	2.229	1.000
		Suwannee	11.531(*)	2.229	7.396E-06
		Ossabaw	7.546(*)	2.243	.013
interoc	Fort Bragg	Fort Campbell	1.154	2.032	1.000
		Fort Rucker	-3.636	2.020	1.000
		Suwannee	.577	2.032	1.000
		Ossabaw	6.995(*)	2.020	.010
		Patuxent	3.226	2.020	1.000
	Fort Campbell	Fort Bragg	-1.154	2.032	1.000
		Fort Rucker	-4.790	2.020	.278
		Suwannee	-.577	2.032	1.000
		Ossabaw	5.841	2.020	.063
		Patuxent	2.073	2.020	1.000
	Fort Rucker	Fort Bragg	3.636	2.020	1.000
		Fort Campbell	4.790	2.020	.278
		Suwannee	4.213	2.020	.571
		Ossabaw	10.631(*)	2.007	4.094E-06
		Patuxent	6.862(*)	2.007	.011
	Suwannee	Fort Bragg	-.577	2.032	1.000
		Fort Campbell	.577	2.032	1.000
		Fort Rucker	-4.213	2.020	.571
		Ossabaw	6.418(*)	2.020	.025
		Patuxent	2.650	2.020	1.000
	Ossabaw	Fort Bragg	-6.995(*)	2.020	.010
		Fort Campbell	-5.841	2.020	.063
		Fort Rucker	-10.631(*)	2.007	4.094E-06
		Suwannee	-6.418(*)	2.020	.025
		Patuxent	-3.769	2.007	.924
	Patuxent	Fort Bragg	-3.226	2.020	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	-2.073	2.020	1.000
		Fort Rucker	-6.862(*)	2.007	.011
		Suwannee	-2.650	2.020	1.000
		Ossabaw	3.769	2.007	.924
labruml	Fort Bragg	Fort Campbell	19.750(*)	4.290	.000
		Fort Rucker	5.077	4.317	1.000
		Suwannee	23.000(*)	4.290	2.979E-06
		Ossabaw	14.750(*)	4.290	.010
		Patuxent	10.750	4.290	.193
	Fort Campbell	Fort Bragg	-19.750(*)	4.290	.000
		Fort Rucker	-14.673(*)	4.317	.012
		Suwannee	3.250	4.290	1.000
		Ossabaw	-5.000	4.290	1.000
		Patuxent	-9.000	4.290	.555
	Fort Rucker	Fort Bragg	-5.077	4.317	1.000
		Fort Campbell	14.673(*)	4.317	.012
		Suwannee	17.923(*)	4.317	.001
		Ossabaw	9.673	4.317	.390
		Patuxent	5.673	4.317	1.000
	Suwannee	Fort Bragg	-23.000(*)	4.290	2.979E-06
		Fort Campbell	-3.250	4.290	1.000
		Fort Rucker	-17.923(*)	4.317	.001
		Ossabaw	-8.250	4.290	.835
		Patuxent	-12.250	4.290	.070
	Ossabaw	Fort Bragg	-14.750(*)	4.290	.010
		Fort Campbell	5.000	4.290	1.000
		Fort Rucker	-9.673	4.317	.390
		Suwannee	8.250	4.290	.835
		Patuxent	-4.000	4.290	1.000
	Patuxent	Fort Bragg	-10.750	4.290	.193
		Fort Campbell	9.000	4.290	.555
		Fort Rucker	-5.673	4.317	1.000
		Suwannee	12.250	4.290	.070
		Ossabaw	4.000	4.290	1.000
palp1len	Fort Bragg	Fort Campbell	1.012	1.369	1.000
		Fort Rucker	-6.975(*)	1.369	1.080E-05
		Suwannee	-2.025	1.369	1.000
		Ossabaw	-5.119(*)	1.369	.003
		Patuxent	-6.413(*)	1.369	7.168E-05
	Fort Campbell	Fort Bragg	-1.012	1.369	1.000
		Fort Rucker	-7.987(*)	1.369	2.685E-07
		Suwannee	-3.038	1.369	.412
		Ossabaw	-6.131(*)	1.369	.000
		Patuxent	-7.425(*)	1.369	2.186E-06
	Fort Rucker	Fort Bragg	6.975(*)	1.369	1.080E-05

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	7.987(*)	1.369	2.685E-07
		Suwannee	4.950(*)	1.369	.006
		Ossabaw	1.856	1.369	1.000
		Patuxent	.563	1.369	1.000
	Suwannee	Fort Bragg	2.025	1.369	1.000
		Fort Campbell	3.038	1.369	.412
		Fort Rucker	-4.950(*)	1.369	.006
		Ossabaw	-3.094	1.369	.371
		Patuxent	-4.387(*)	1.369	.023
	Ossabaw	Fort Bragg	5.119(*)	1.369	.003
		Fort Campbell	6.131(*)	1.369	.000
		Fort Rucker	-1.856	1.369	1.000
		Suwannee	3.094	1.369	.371
		Patuxent	-1.294	1.369	1.000
	Patuxent	Fort Bragg	6.413(*)	1.369	7.168E-05
		Fort Campbell	7.425(*)	1.369	2.186E-06
		Fort Rucker	-.563	1.369	1.000
		Suwannee	4.387(*)	1.369	.023
		Ossabaw	1.294	1.369	1.000
palp1wid	Fort Bragg	Fort Campbell	1.969	.873	.375
		Fort Rucker	-1.013	.873	1.000
		Suwannee	-1.969	.873	.375
		Ossabaw	.450	.873	1.000
		Patuxent	-.169	.873	1.000
	Fort Campbell	Fort Bragg	-1.969	.873	.375
		Fort Rucker	-2.981(*)	.873	.011
		Suwannee	-3.938(*)	.873	.000
		Ossabaw	-1.519	.873	1.000
		Patuxent	-2.138	.873	.225
	Fort Rucker	Fort Bragg	1.013	.873	1.000
		Fort Campbell	2.981(*)	.873	.011
		Suwannee	-.956	.873	1.000
		Ossabaw	1.463	.873	1.000
		Patuxent	.844	.873	1.000
	Suwannee	Fort Bragg	1.969	.873	.375
		Fort Campbell	3.938(*)	.873	.000
		Fort Rucker	.956	.873	1.000
		Ossabaw	2.419	.873	.090
		Patuxent	1.800	.873	.603
	Ossabaw	Fort Bragg	-.450	.873	1.000
		Fort Campbell	1.519	.873	1.000
		Fort Rucker	-1.463	.873	1.000
		Suwannee	-2.419	.873	.090
		Patuxent	-.619	.873	1.000
	Patuxent	Fort Bragg	.169	.873	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	2.138	.873	.225
		Fort Rucker	-.844	.873	1.000
		Suwannee	-1.800	.873	.603
		Ossabaw	.619	.873	1.000
palp1rat	Fort Bragg	Fort Campbell	-.088	.067	1.000
		Fort Rucker	-.170	.067	.175
		Suwannee	.036	.067	1.000
		Ossabaw	-.205(*)	.067	.036
		Patuxent	-.191	.067	.069
	Fort Campbell	Fort Bragg	.088	.067	1.000
		Fort Rucker	-.082	.067	1.000
		Suwannee	.124	.067	.970
		Ossabaw	-.117	.067	1.000
		Patuxent	-.103	.067	1.000
	Fort Rucker	Fort Bragg	.170	.067	.175
		Fort Campbell	.082	.067	1.000
		Suwannee	.206(*)	.067	.035
		Ossabaw	-.035	.067	1.000
		Patuxent	-.022	.067	1.000
	Suwannee	Fort Bragg	-.036	.067	1.000
		Fort Campbell	-.124	.067	.970
		Fort Rucker	-.206(*)	.067	.035
		Ossabaw	-.241(*)	.067	.006
		Patuxent	-.227(*)	.067	.012
	Ossabaw	Fort Bragg	.205(*)	.067	.036
		Fort Campbell	.117	.067	1.000
		Fort Rucker	.035	.067	1.000
		Suwannee	.241(*)	.067	.006
		Patuxent	.014	.067	1.000
	Patuxent	Fort Bragg	.191	.067	.069
		Fort Campbell	.103	.067	1.000
		Fort Rucker	.022	.067	1.000
		Suwannee	.227(*)	.067	.012
		Ossabaw	-.014	.067	1.000
palp2len	Fort Bragg	Fort Campbell	5.758	2.011	.069
		Fort Rucker	.288	2.023	1.000
		Suwannee	.189	2.011	1.000
		Ossabaw	.750	2.023	1.000
		Patuxent	-.205	2.011	1.000
	Fort Campbell	Fort Bragg	-5.758	2.011	.069
		Fort Rucker	-5.469	2.011	.105
		Suwannee	-5.569	1.998	.086
		Ossabaw	-5.008	2.011	.202
		Patuxent	-5.962(*)	1.998	.047
	Fort Rucker	Fort Bragg	-.288	2.023	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	5.469	2.011	.105
		Suwannee	-.100	2.011	1.000
		Ossabaw	.462	2.023	1.000
		Patuxent	-.493	2.011	1.000
	Suwannee	Fort Bragg	-.189	2.011	1.000
		Fort Campbell	5.569	1.998	.086
		Fort Rucker	.100	2.011	1.000
		Ossabaw	.561	2.011	1.000
		Patuxent	-.394	1.998	1.000
	Ossabaw	Fort Bragg	-.750	2.023	1.000
		Fort Campbell	5.008	2.011	.202
		Fort Rucker	-.462	2.023	1.000
		Suwannee	-.561	2.011	1.000
		Patuxent	-.955	2.011	1.000
	Patuxent	Fort Bragg	.205	2.011	1.000
		Fort Campbell	5.962(*)	1.998	.047
		Fort Rucker	.493	2.011	1.000
		Suwannee	.394	1.998	1.000
		Ossabaw	.955	2.011	1.000
palp2wid	Fort Bragg	Fort Campbell	2.189	.837	.142
		Fort Rucker	-2.367	.837	.076
		Suwannee	-2.479	.837	.050
		Ossabaw	-.981	.842	1.000
		Patuxent	-1.354	.837	1.000
	Fort Campbell	Fort Bragg	-2.189	.837	.142
		Fort Rucker	-4.556(*)	.831	1.658E-06
		Suwannee	-4.669(*)	.831	8.374E-07
		Ossabaw	-3.170(*)	.837	.003
		Patuxent	-3.544(*)	.831	.000
	Fort Rucker	Fort Bragg	2.367	.837	.076
		Fort Campbell	4.556(*)	.831	1.658E-06
		Suwannee	-.112	.831	1.000
		Ossabaw	1.386	.837	1.000
		Patuxent	1.013	.831	1.000
	Suwannee	Fort Bragg	2.479	.837	.050
		Fort Campbell	4.669(*)	.831	8.374E-07
		Fort Rucker	.112	.831	1.000
		Ossabaw	1.499	.837	1.000
		Patuxent	1.125	.831	1.000
	Ossabaw	Fort Bragg	.981	.842	1.000
		Fort Campbell	3.170(*)	.837	.003
		Fort Rucker	-1.386	.837	1.000
		Suwannee	-1.499	.837	1.000
		Patuxent	-.374	.837	1.000
	Patuxent	Fort Bragg	1.354	.837	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	3.544(*)	.831	.000
		Fort Rucker	-1.013	.831	1.000
		Suwannee	-1.125	.831	1.000
		Ossabaw	.374	.837	1.000
palp2rat	Fort Bragg	Fort Campbell	-.334	.166	.680
		Fort Rucker	.432	.167	.154
		Suwannee	.540(*)	.166	.019
		Ossabaw	.233	.167	1.000
		Patuxent	.283	.166	1.000
	Fort Campbell	Fort Bragg	.334	.166	.680
		Fort Rucker	.766(*)	.166	9.709E-05
		Suwannee	.874(*)	.165	3.996E-06
		Ossabaw	.566(*)	.166	.011
		Patuxent	.617(*)	.165	.003
	Fort Rucker	Fort Bragg	-.432	.167	.154
		Fort Campbell	-.766(*)	.166	9.709E-05
		Suwannee	.108	.166	1.000
		Ossabaw	-.199	.167	1.000
		Patuxent	-.149	.166	1.000
	Suwannee	Fort Bragg	-.540(*)	.166	.019
		Fort Campbell	-.874(*)	.165	3.996E-06
		Fort Rucker	-.108	.166	1.000
		Ossabaw	-.308	.166	.973
		Patuxent	-.257	.165	1.000
	Ossabaw	Fort Bragg	-.233	.167	1.000
		Fort Campbell	-.566(*)	.166	.011
		Fort Rucker	.199	.167	1.000
		Suwannee	.308	.166	.973
		Patuxent	.051	.166	1.000
	Patuxent	Fort Bragg	-.283	.166	1.000
		Fort Campbell	-.617(*)	.165	.003
		Fort Rucker	.149	.166	1.000
		Suwannee	.257	.165	1.000
		Ossabaw	-.051	.166	1.000
palp3len	Fort Bragg	Fort Campbell	7.212(*)	2.143	.013
		Fort Rucker	1.442	2.143	1.000
		Suwannee	6.865(*)	2.143	.023
		Ossabaw	3.923	2.143	1.000
		Patuxent	.000	2.143	1.000
	Fort Campbell	Fort Bragg	-7.212(*)	2.143	.013
		Fort Rucker	-5.769	2.143	.114
		Suwannee	-.346	2.143	1.000
		Ossabaw	-3.288	2.143	1.000
		Patuxent	-7.212(*)	2.143	.013
	Fort Rucker	Fort Bragg	-1.442	2.143	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	5.769	2.143	.114
		Suwannee	5.423	2.143	.181
		Ossabaw	2.481	2.143	1.000
		Patuxent	-1.442	2.143	1.000
	Suwannee	Fort Bragg	-6.865(*)	2.143	.023
		Fort Campbell	.346	2.143	1.000
		Fort Rucker	-5.423	2.143	.181
		Ossabaw	-2.942	2.143	1.000
		Patuxent	-6.865(*)	2.143	.023
	Ossabaw	Fort Bragg	-3.923	2.143	1.000
		Fort Campbell	3.288	2.143	1.000
		Fort Rucker	-2.481	2.143	1.000
		Suwannee	2.942	2.143	1.000
		Patuxent	-3.923	2.143	1.000
	Patuxent	Fort Bragg	.000	2.143	1.000
		Fort Campbell	7.212(*)	2.143	.013
		Fort Rucker	1.442	2.143	1.000
		Suwannee	6.865(*)	2.143	.023
		Ossabaw	3.923	2.143	1.000
palp3wid	Fort Bragg	Fort Campbell	.865	1.086	1.000
		Fort Rucker	-2.637	1.079	.229
		Suwannee	-3.923(*)	1.086	.006
		Ossabaw	-.692	1.086	1.000
		Patuxent	-3.346(*)	1.086	.035
	Fort Campbell	Fort Bragg	-.865	1.086	1.000
		Fort Rucker	-3.502(*)	1.079	.020
		Suwannee	-4.788(*)	1.086	.000
		Ossabaw	-1.558	1.086	1.000
		Patuxent	-4.212(*)	1.086	.002
	Fort Rucker	Fort Bragg	2.637	1.079	.229
		Fort Campbell	3.502(*)	1.079	.020
		Suwannee	-1.287	1.079	1.000
		Ossabaw	1.944	1.079	1.000
		Patuxent	-.710	1.079	1.000
	Suwannee	Fort Bragg	3.923(*)	1.086	.006
		Fort Campbell	4.788(*)	1.086	.000
		Fort Rucker	1.287	1.079	1.000
		Ossabaw	3.231(*)	1.086	.048
		Patuxent	.577	1.086	1.000
	Ossabaw	Fort Bragg	.692	1.086	1.000
		Fort Campbell	1.558	1.086	1.000
		Fort Rucker	-1.944	1.079	1.000
		Suwannee	-3.231(*)	1.086	.048
		Patuxent	-2.654	1.086	.229
	Patuxent	Fort Bragg	3.346(*)	1.086	.035

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	4.212(*)	1.086	.002
		Fort Rucker	.710	1.079	1.000
		Suwannee	-.577	1.086	1.000
		Ossabaw	2.654	1.086	.229
palp3rat	Fort Bragg	Fort Campbell	.059	.162	1.000
		Fort Rucker	.368	.162	.360
		Suwannee	.737(*)	.162	.000
		Ossabaw	.179	.162	1.000
		Patuxent	.496(*)	.162	.037
	Fort Campbell	Fort Bragg	-.059	.162	1.000
		Fort Rucker	.308	.162	.872
		Suwannee	.677(*)	.162	.001
		Ossabaw	.120	.162	1.000
		Patuxent	.437	.162	.113
	Fort Rucker	Fort Bragg	-.368	.162	.360
		Fort Campbell	-.308	.162	.872
		Suwannee	.369	.162	.355
		Ossabaw	-.189	.162	1.000
		Patuxent	.128	.162	1.000
	Suwannee	Fort Bragg	-.737(*)	.162	.000
		Fort Campbell	-.677(*)	.162	.001
		Fort Rucker	-.369	.162	.355
		Ossabaw	-.558(*)	.162	.010
		Patuxent	-.241	.162	1.000
	Ossabaw	Fort Bragg	-.179	.162	1.000
		Fort Campbell	-.120	.162	1.000
		Fort Rucker	.189	.162	1.000
		Suwannee	.558(*)	.162	.010
		Patuxent	.317	.162	.771
	Patuxent	Fort Bragg	-.496(*)	.162	.037
		Fort Campbell	-.437	.162	.113
		Fort Rucker	-.128	.162	1.000
		Suwannee	.241	.162	1.000
		Ossabaw	-.317	.162	.771
palp4len	Fort Bragg	Fort Campbell	3.149	1.356	.317
		Fort Rucker	2.860	1.356	.540
		Suwannee	4.322(*)	1.365	.026
		Ossabaw	2.572	1.356	.887
		Patuxent	4.014	1.356	.051
	Fort Campbell	Fort Bragg	-3.149	1.356	.317
		Fort Rucker	-.288	1.347	1.000
		Suwannee	1.174	1.356	1.000
		Ossabaw	-.577	1.347	1.000
		Patuxent	.865	1.347	1.000
	Fort Rucker	Fort Bragg	-2.860	1.356	.540

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	.288	1.347	1.000
		Suwannee	1.462	1.356	1.000
		Ossabaw	-.288	1.347	1.000
		Patuxent	1.154	1.347	1.000
	Suwannee	Fort Bragg	-4.322(*)	1.365	.026
		Fort Campbell	-1.174	1.356	1.000
		Fort Rucker	-1.462	1.356	1.000
		Ossabaw	-1.751	1.356	1.000
		Patuxent	-.308	1.356	1.000
	Ossabaw	Fort Bragg	-2.572	1.356	.887
		Fort Campbell	.577	1.347	1.000
		Fort Rucker	.288	1.347	1.000
		Suwannee	1.751	1.356	1.000
		Patuxent	1.442	1.347	1.000
	Patuxent	Fort Bragg	-4.014	1.356	.051
		Fort Campbell	-.865	1.347	1.000
		Fort Rucker	-1.154	1.347	1.000
		Suwannee	.308	1.356	1.000
		Ossabaw	-1.442	1.347	1.000
palp4wid	Fort Bragg	Fort Campbell	1.101	.678	1.000
		Fort Rucker	-.284	.678	1.000
		Suwannee	-1.243	.682	1.000
		Ossabaw	.293	.678	1.000
		Patuxent	-.399	.678	1.000
	Fort Campbell	Fort Bragg	-1.101	.678	1.000
		Fort Rucker	-1.385	.673	.614
		Suwannee	-2.344(*)	.678	.010
		Ossabaw	-.808	.673	1.000
		Patuxent	-1.500	.673	.403
	Fort Rucker	Fort Bragg	.284	.678	1.000
		Fort Campbell	1.385	.673	.614
		Suwannee	-.960	.678	1.000
		Ossabaw	.577	.673	1.000
		Patuxent	-.115	.673	1.000
	Suwannee	Fort Bragg	1.243	.682	1.000
		Fort Campbell	2.344(*)	.678	.010
		Fort Rucker	.960	.678	1.000
		Ossabaw	1.536	.678	.365
		Patuxent	.844	.678	1.000
	Ossabaw	Fort Bragg	-.293	.678	1.000
		Fort Campbell	.808	.673	1.000
		Fort Rucker	-.577	.673	1.000
		Suwannee	-1.536	.678	.365
		Patuxent	-.692	.673	1.000
	Patuxent	Fort Bragg	.399	.678	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	1.500	.673	.403
		Fort Rucker	.115	.673	1.000
		Suwannee	-.844	.678	1.000
		Ossabaw	.692	.673	1.000
palp4rat	Fort Bragg	Fort Campbell	-.046	.114	1.000
		Fort Rucker	.116	.114	1.000
		Suwannee	.325	.115	.075
		Ossabaw	.032	.114	1.000
		Patuxent	.208	.114	1.000
	Fort Campbell	Fort Bragg	.046	.114	1.000
		Fort Rucker	.162	.113	1.000
		Suwannee	.371(*)	.114	.020
		Ossabaw	.078	.113	1.000
		Patuxent	.254	.113	.390
	Fort Rucker	Fort Bragg	-.116	.114	1.000
		Fort Campbell	-.162	.113	1.000
		Suwannee	.209	.114	1.000
		Ossabaw	-.084	.113	1.000
		Patuxent	.092	.113	1.000
	Suwannee	Fort Bragg	-.325	.115	.075
		Fort Campbell	-.371(*)	.114	.020
		Fort Rucker	-.209	.114	1.000
		Ossabaw	-.293	.114	.163
		Patuxent	-.117	.114	1.000
	Ossabaw	Fort Bragg	-.032	.114	1.000
		Fort Campbell	-.078	.113	1.000
		Fort Rucker	.084	.113	1.000
		Suwannee	.293	.114	.163
		Patuxent	.176	.113	1.000
	Patuxent	Fort Bragg	-.208	.114	1.000
		Fort Campbell	-.254	.113	.390
		Fort Rucker	-.092	.113	1.000
		Suwannee	.117	.114	1.000
		Ossabaw	-.176	.113	1.000
flag1len	Fort Bragg	Fort Campbell	12.500	4.878	.165
		Fort Rucker	5.250	4.878	1.000
		Suwannee	4.500	4.878	1.000
		Ossabaw	13.000	4.878	.123
		Patuxent	5.250	4.878	1.000
	Fort Campbell	Fort Bragg	-12.500	4.878	.165
		Fort Rucker	-7.250	4.878	1.000
		Suwannee	-8.000	4.878	1.000
		Ossabaw	.500	4.878	1.000
		Patuxent	-7.250	4.878	1.000
	Fort Rucker	Fort Bragg	-5.250	4.878	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	7.250	4.878	1.000
		Suwannee	-.750	4.878	1.000
		Ossabaw	7.750	4.878	1.000
		Patuxent	.000	4.878	1.000
	Suwannee	Fort Bragg	-4.500	4.878	1.000
		Fort Campbell	8.000	4.878	1.000
		Fort Rucker	.750	4.878	1.000
		Ossabaw	8.500	4.878	1.000
		Patuxent	.750	4.878	1.000
	Ossabaw	Fort Bragg	-13.000	4.878	.123
		Fort Campbell	-.500	4.878	1.000
		Fort Rucker	-7.750	4.878	1.000
		Suwannee	-8.500	4.878	1.000
		Patuxent	-7.750	4.878	1.000
	Patuxent	Fort Bragg	-5.250	4.878	1.000
		Fort Campbell	7.250	4.878	1.000
		Fort Rucker	.000	4.878	1.000
		Suwannee	-.750	4.878	1.000
		Ossabaw	7.750	4.878	1.000
flag1wid	Fort Bragg	Fort Campbell	-1.350	.871	1.000
		Fort Rucker	-3.656(*)	.871	.001
		Suwannee	-3.488(*)	.871	.001
		Ossabaw	-1.406	.871	1.000
		Patuxent	-1.800	.871	.597
	Fort Campbell	Fort Bragg	1.350	.871	1.000
		Fort Rucker	-2.306	.871	.129
		Suwannee	-2.137	.871	.222
		Ossabaw	-.056	.871	1.000
		Patuxent	-.450	.871	1.000
	Fort Rucker	Fort Bragg	3.656(*)	.871	.001
		Fort Campbell	2.306	.871	.129
		Suwannee	.169	.871	1.000
		Ossabaw	2.250	.871	.156
		Patuxent	1.856	.871	.511
	Suwannee	Fort Bragg	3.488(*)	.871	.001
		Fort Campbell	2.137	.871	.222
		Fort Rucker	-.169	.871	1.000
		Ossabaw	2.081	.871	.264
		Patuxent	1.688	.871	.807
	Ossabaw	Fort Bragg	1.406	.871	1.000
		Fort Campbell	.056	.871	1.000
		Fort Rucker	-2.250	.871	.156
		Suwannee	-2.081	.871	.264
		Patuxent	-.394	.871	1.000
	Patuxent	Fort Bragg	1.800	.871	.597

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	.450	.871	1.000
		Fort Rucker	-1.856	.871	.511
		Suwannee	-1.688	.871	.807
		Ossabaw	.394	.871	1.000
flag1rat	Fort Bragg	Fort Campbell	1.158	.443	.142
		Fort Rucker	1.801(*)	.443	.001
		Suwannee	1.844(*)	.443	.001
		Ossabaw	1.166	.443	.136
		Patuxent	1.138	.443	.162
	Fort Campbell	Fort Bragg	-1.158	.443	.142
		Fort Rucker	.642	.443	1.000
		Suwannee	.685	.443	1.000
		Ossabaw	.008	.443	1.000
		Patuxent	-.021	.443	1.000
	Fort Rucker	Fort Bragg	-1.801(*)	.443	.001
		Fort Campbell	-.642	.443	1.000
		Suwannee	.043	.443	1.000
		Ossabaw	-.635	.443	1.000
		Patuxent	-.663	.443	1.000
	Suwannee	Fort Bragg	-1.844(*)	.443	.001
		Fort Campbell	-.685	.443	1.000
		Fort Rucker	-.043	.443	1.000
		Ossabaw	-.678	.443	1.000
		Patuxent	-.706	.443	1.000
	Ossabaw	Fort Bragg	-1.166	.443	.136
		Fort Campbell	-.008	.443	1.000
		Fort Rucker	.635	.443	1.000
		Suwannee	.678	.443	1.000
		Patuxent	-.028	.443	1.000
	Patuxent	Fort Bragg	-1.138	.443	.162
		Fort Campbell	.021	.443	1.000
		Fort Rucker	.663	.443	1.000
		Suwannee	.706	.443	1.000
		Ossabaw	.028	.443	1.000
flag2len	Fort Bragg	Fort Campbell	-2.306	1.534	1.000
		Fort Rucker	-3.375	1.534	.431
		Suwannee	-4.219	1.534	.096
		Ossabaw	.675	1.534	1.000
		Patuxent	-2.138	1.534	1.000
	Fort Campbell	Fort Bragg	2.306	1.534	1.000
		Fort Rucker	-1.069	1.534	1.000
		Suwannee	-1.912	1.534	1.000
		Ossabaw	2.981	1.534	.797
		Patuxent	.169	1.534	1.000
	Fort Rucker	Fort Bragg	3.375	1.534	.431

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	1.069	1.534	1.000
		Suwannee	-.844	1.534	1.000
		Ossabaw	4.050	1.534	.133
		Patuxent	1.237	1.534	1.000
	Suwannee	Fort Bragg	4.219	1.534	.096
		Fort Campbell	1.912	1.534	1.000
		Fort Rucker	.844	1.534	1.000
		Ossabaw	4.894(*)	1.534	.024
		Patuxent	2.081	1.534	1.000
	Ossabaw	Fort Bragg	-.675	1.534	1.000
		Fort Campbell	-2.981	1.534	.797
		Fort Rucker	-4.050	1.534	.133
		Suwannee	-4.894(*)	1.534	.024
		Patuxent	-2.813	1.534	1.000
	Patuxent	Fort Bragg	2.138	1.534	1.000
		Fort Campbell	-.169	1.534	1.000
		Fort Rucker	-1.237	1.534	1.000
		Suwannee	-2.081	1.534	1.000
		Ossabaw	2.813	1.534	1.000
flag2wid	Fort Bragg	Fort Campbell	.844	.571	1.000
		Fort Rucker	-.113	.571	1.000
		Suwannee	-.506	.571	1.000
		Ossabaw	.000	.571	1.000
		Patuxent	1.069	.571	.934
	Fort Campbell	Fort Bragg	-.844	.571	1.000
		Fort Rucker	-.956	.571	1.000
		Suwannee	-1.350	.571	.282
		Ossabaw	-.844	.571	1.000
		Patuxent	.225	.571	1.000
	Fort Rucker	Fort Bragg	.113	.571	1.000
		Fort Campbell	.956	.571	1.000
		Suwannee	-.394	.571	1.000
		Ossabaw	.113	.571	1.000
		Patuxent	1.181	.571	.593
	Suwannee	Fort Bragg	.506	.571	1.000
		Fort Campbell	1.350	.571	.282
		Fort Rucker	.394	.571	1.000
		Ossabaw	.506	.571	1.000
		Patuxent	1.575	.571	.093
	Ossabaw	Fort Bragg	.000	.571	1.000
		Fort Campbell	.844	.571	1.000
		Fort Rucker	-.113	.571	1.000
		Suwannee	-.506	.571	1.000
		Patuxent	1.069	.571	.934
	Patuxent	Fort Bragg	-1.069	.571	.934

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	-.225	.571	1.000
		Fort Rucker	-1.181	.571	.593
		Suwannee	-1.575	.571	.093
		Ossabaw	-1.069	.571	.934
flag2rat	Fort Bragg	Fort Campbell	-.328	.149	.430
		Fort Rucker	-.144	.149	1.000
		Suwannee	-.096	.149	1.000
		Ossabaw	-.013	.149	1.000
		Patuxent	-.400	.149	.116
	Fort Campbell	Fort Bragg	.328	.149	.430
		Fort Rucker	.183	.149	1.000
		Suwannee	.231	.149	1.000
		Ossabaw	.315	.149	.529
		Patuxent	-.072	.149	1.000
	Fort Rucker	Fort Bragg	.144	.149	1.000
		Fort Campbell	-.183	.149	1.000
		Suwannee	.048	.149	1.000
		Ossabaw	.131	.149	1.000
		Patuxent	-.255	.149	1.000
	Suwannee	Fort Bragg	.096	.149	1.000
		Fort Campbell	-.231	.149	1.000
		Fort Rucker	-.048	.149	1.000
		Ossabaw	.084	.149	1.000
		Patuxent	-.303	.149	.640
	Ossabaw	Fort Bragg	.013	.149	1.000
		Fort Campbell	-.315	.149	.529
		Fort Rucker	-.131	.149	1.000
		Suwannee	-.084	.149	1.000
		Patuxent	-.387	.149	.148
	Patuxent	Fort Bragg	.400	.149	.116
		Fort Campbell	.072	.149	1.000
		Fort Rucker	.255	.149	1.000
		Suwannee	.303	.149	.640
		Ossabaw	.387	.149	.148
flag3len	Fort Bragg	Fort Campbell	-1.744	1.575	1.000
		Fort Rucker	-4.162	1.575	.132
		Suwannee	-5.906(*)	1.575	.003
		Ossabaw	.225	1.575	1.000
		Patuxent	-2.700	1.575	1.000
	Fort Campbell	Fort Bragg	1.744	1.575	1.000
		Fort Rucker	-2.419	1.575	1.000
		Suwannee	-4.163	1.575	.132
		Ossabaw	1.969	1.575	1.000
		Patuxent	-.956	1.575	1.000
	Fort Rucker	Fort Bragg	4.162	1.575	.132

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	2.419	1.575	1.000
		Suwannee	-1.744	1.575	1.000
		Ossabaw	4.388	1.575	.087
		Patuxent	1.463	1.575	1.000
	Suwannee	Fort Bragg	5.906(*)	1.575	.003
		Fort Campbell	4.163	1.575	.132
		Fort Rucker	1.744	1.575	1.000
		Ossabaw	6.131(*)	1.575	.002
		Patuxent	3.206	1.575	.644
	Ossabaw	Fort Bragg	-.225	1.575	1.000
		Fort Campbell	-1.969	1.575	1.000
		Fort Rucker	-4.388	1.575	.087
		Suwannee	-6.131(*)	1.575	.002
		Patuxent	-2.925	1.575	.968
	Patuxent	Fort Bragg	2.700	1.575	1.000
		Fort Campbell	.956	1.575	1.000
		Fort Rucker	-1.463	1.575	1.000
		Suwannee	-3.206	1.575	.644
		Ossabaw	2.925	1.575	.968
flag3wid	Fort Bragg	Fort Campbell	1.631(*)	.502	.020
		Fort Rucker	.225	.502	1.000
		Suwannee	-.281	.502	1.000
		Ossabaw	.337	.502	1.000
		Patuxent	1.012	.502	.676
	Fort Campbell	Fort Bragg	-1.631(*)	.502	.020
		Fort Rucker	-1.406	.502	.083
		Suwannee	-1.912(*)	.502	.003
		Ossabaw	-1.294	.502	.160
		Patuxent	-.619	.502	1.000
	Fort Rucker	Fort Bragg	-.225	.502	1.000
		Fort Campbell	1.406	.502	.083
		Suwannee	-.506	.502	1.000
		Ossabaw	.113	.502	1.000
		Patuxent	.788	.502	1.000
	Suwannee	Fort Bragg	.281	.502	1.000
		Fort Campbell	1.912(*)	.502	.003
		Fort Rucker	.506	.502	1.000
		Ossabaw	.619	.502	1.000
		Patuxent	1.294	.502	.160
	Ossabaw	Fort Bragg	-.337	.502	1.000
		Fort Campbell	1.294	.502	.160
		Fort Rucker	-.113	.502	1.000
		Suwannee	-.619	.502	1.000
		Patuxent	.675	.502	1.000
	Patuxent	Fort Bragg	-1.012	.502	.676

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	.619	.502	1.000
		Fort Rucker	-.788	.502	1.000
		Suwannee	-1.294	.502	.160
		Ossabaw	-.675	.502	1.000
flag3rat	Fort Bragg	Fort Campbell	-.533(*)	.148	.006
		Fort Rucker	-.276	.148	.936
		Suwannee	-.273	.148	.982
		Ossabaw	-.103	.148	1.000
		Patuxent	-.419	.148	.073
	Fort Campbell	Fort Bragg	.533(*)	.148	.006
		Fort Rucker	.257	.148	1.000
		Suwannee	.260	.148	1.000
		Ossabaw	.430	.148	.058
		Patuxent	.114	.148	1.000
	Fort Rucker	Fort Bragg	.276	.148	.936
		Fort Campbell	-.257	.148	1.000
		Suwannee	.003	.148	1.000
		Ossabaw	.173	.148	1.000
		Patuxent	-.143	.148	1.000
	Suwannee	Fort Bragg	.273	.148	.982
		Fort Campbell	-.260	.148	1.000
		Fort Rucker	-.003	.148	1.000
		Ossabaw	.170	.148	1.000
		Patuxent	-.146	.148	1.000
	Ossabaw	Fort Bragg	.103	.148	1.000
		Fort Campbell	-.430	.148	.058
		Fort Rucker	-.173	.148	1.000
		Suwannee	-.170	.148	1.000
		Patuxent	-.317	.148	.492
	Patuxent	Fort Bragg	.419	.148	.073
		Fort Campbell	-.114	.148	1.000
		Fort Rucker	.143	.148	1.000
		Suwannee	.146	.148	1.000
		Ossabaw	.317	.148	.492
flag4len	Fort Bragg	Fort Campbell	-1.912	1.465	1.000
		Fort Rucker	-3.319	1.465	.366
		Suwannee	-4.612(*)	1.465	.028
		Ossabaw	2.138	1.465	1.000
		Patuxent	-1.237	1.465	1.000
	Fort Campbell	Fort Bragg	1.912	1.465	1.000
		Fort Rucker	-1.406	1.465	1.000
		Suwannee	-2.700	1.465	1.000
		Ossabaw	4.050	1.465	.092
		Patuxent	.675	1.465	1.000
	Fort Rucker	Fort Bragg	3.319	1.465	.366

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
		Fort Campbell	1.406	1.465	1.000
		Suwannee	-1.294	1.465	1.000
		Ossabaw	5.456(*)	1.465	.004
		Patuxent	2.081	1.465	1.000
	Suwannee	Fort Bragg	4.612(*)	1.465	.028
		Fort Campbell	2.700	1.465	1.000
		Fort Rucker	1.294	1.465	1.000
		Ossabaw	6.750(*)	1.465	.000
		Patuxent	3.375	1.465	.332
	Ossabaw	Fort Bragg	-2.138	1.465	1.000
		Fort Campbell	-4.050	1.465	.092
		Fort Rucker	-5.456(*)	1.465	.004
		Suwannee	-6.750(*)	1.465	.000
		Patuxent	-3.375	1.465	.332
	Patuxent	Fort Bragg	1.237	1.465	1.000
		Fort Campbell	-.675	1.465	1.000
		Fort Rucker	-2.081	1.465	1.000
		Suwannee	-3.375	1.465	.332
		Ossabaw	3.375	1.465	.332
flag4wid	Fort Bragg	Fort Campbell	1.181	.490	.251
		Fort Rucker	.337	.490	1.000
		Suwannee	.225	.490	1.000
		Ossabaw	.619	.490	1.000
		Patuxent	.900	.490	1.000
	Fort Campbell	Fort Bragg	-1.181	.490	.251
		Fort Rucker	-.844	.490	1.000
		Suwannee	-.956	.490	.785
		Ossabaw	-.563	.490	1.000
		Patuxent	-.281	.490	1.000
	Fort Rucker	Fort Bragg	-.337	.490	1.000
		Fort Campbell	.844	.490	1.000
		Suwannee	-.112	.490	1.000
		Ossabaw	.281	.490	1.000
		Patuxent	.563	.490	1.000
	Suwannee	Fort Bragg	-.225	.490	1.000
		Fort Campbell	.956	.490	.785
		Fort Rucker	.112	.490	1.000
		Ossabaw	.394	.490	1.000
		Patuxent	.675	.490	1.000
	Ossabaw	Fort Bragg	-.619	.490	1.000
		Fort Campbell	.563	.490	1.000
		Fort Rucker	-.281	.490	1.000
		Suwannee	-.394	.490	1.000
		Patuxent	.281	.490	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Patuxent	Fort Bragg	-.900	.490	1.000
		Fort Campbell	.281	.490	1.000
		Fort Rucker	-.563	.490	1.000
		Suwannee	-.675	.490	1.000
		Ossabaw	-.281	.490	1.000
flag4rat	Fort Bragg	Fort Campbell	-.418	.147	.072
		Fort Rucker	-.282	.147	.835
		Suwannee	-.314	.147	.504
		Ossabaw	-.109	.147	1.000
		Patuxent	-.322	.147	.438
	Fort Campbell	Fort Bragg	.418	.147	.072
		Fort Rucker	.136	.147	1.000
		Suwannee	.104	.147	1.000
		Ossabaw	.309	.147	.547
		Patuxent	.096	.147	1.000
	Fort Rucker	Fort Bragg	.282	.147	.835
		Fort Campbell	-.136	.147	1.000
		Suwannee	-.032	.147	1.000
		Ossabaw	.173	.147	1.000
		Patuxent	-.040	.147	1.000
	Suwannee	Fort Bragg	.314	.147	.504
		Fort Campbell	-.104	.147	1.000
		Fort Rucker	.032	.147	1.000
		Ossabaw	.204	.147	1.000
		Patuxent	-.008	.147	1.000
	Ossabaw	Fort Bragg	.109	.147	1.000
		Fort Campbell	-.309	.147	.547
		Fort Rucker	-.173	.147	1.000
		Suwannee	-.204	.147	1.000
		Patuxent	-.213	.147	1.000
	Patuxent	Fort Bragg	.322	.147	.438
		Fort Campbell	-.096	.147	1.000
		Fort Rucker	.040	.147	1.000
		Suwannee	.008	.147	1.000
		Ossabaw	.213	.147	1.000
flag5len	Fort Bragg	Fort Campbell	-1.180	1.479	1.000
		Fort Rucker	-2.981	1.470	.655
		Suwannee	-5.344(*)	1.470	.005
		Ossabaw	3.037	1.470	.598
		Patuxent	-1.013	1.470	1.000
	Fort Campbell	Fort Bragg	1.180	1.479	1.000
		Fort Rucker	-1.801	1.479	1.000
		Suwannee	-4.164	1.479	.079
		Ossabaw	4.217	1.479	.071
		Patuxent	.167	1.479	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Fort Rucker	Fort Bragg	2.981	1.470	.655
		Fort Campbell	1.801	1.479	1.000
		Suwannee	-2.362	1.470	1.000
		Ossabaw	6.019(*)	1.470	.001
		Patuxent	1.969	1.470	1.000
	Suwannee	Fort Bragg	5.344(*)	1.470	.005
		Fort Campbell	4.164	1.479	.079
		Fort Rucker	2.362	1.470	1.000
		Ossabaw	8.381(*)	1.470	5.355E-07
		Patuxent	4.331	1.470	.053
	Ossabaw	Fort Bragg	-3.037	1.470	.598
		Fort Campbell	-4.217	1.479	.071
		Fort Rucker	-6.019(*)	1.470	.001
		Suwannee	-8.381(*)	1.470	5.355E-07
		Patuxent	-4.050	1.470	.095
	Patuxent	Fort Bragg	1.013	1.470	1.000
		Fort Campbell	-.167	1.479	1.000
		Fort Rucker	-1.969	1.470	1.000
		Suwannee	-4.331	1.470	.053
		Ossabaw	4.050	1.470	.095
flag5wid	Fort Bragg	Fort Campbell	.775	.497	1.000
		Fort Rucker	.281	.494	1.000
		Suwannee	-.113	.494	1.000
		Ossabaw	.675	.494	1.000
		Patuxent	.619	.494	1.000
	Fort Campbell	Fort Bragg	-.775	.497	1.000
		Fort Rucker	-.493	.497	1.000
		Suwannee	-.887	.497	1.000
		Ossabaw	-.100	.497	1.000
		Patuxent	-.156	.497	1.000
	Fort Rucker	Fort Bragg	-.281	.494	1.000
		Fort Campbell	.493	.497	1.000
		Suwannee	-.394	.494	1.000
		Ossabaw	.394	.494	1.000
		Patuxent	.337	.494	1.000
	Suwannee	Fort Bragg	.113	.494	1.000
		Fort Campbell	.887	.497	1.000
		Fort Rucker	.394	.494	1.000
		Ossabaw	.788	.494	1.000
		Patuxent	.731	.494	1.000
	Ossabaw	Fort Bragg	-.675	.494	1.000
		Fort Campbell	.100	.497	1.000
		Fort Rucker	-.394	.494	1.000
		Suwannee	-.788	.494	1.000
		Patuxent	-.056	.494	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Patuxent	Fort Bragg	-.619	.494	1.000
		Fort Campbell	.156	.497	1.000
		Fort Rucker	-.337	.494	1.000
		Suwannee	-.731	.494	1.000
		Ossabaw	.056	.494	1.000
flag5rat	Fort Bragg	Fort Campbell	-.283	.149	.886
		Fort Rucker	-.278	.148	.934
		Suwannee	-.299	.148	.676
		Ossabaw	-.078	.148	1.000
		Patuxent	-.266	.148	1.000
	Fort Campbell	Fort Bragg	.283	.149	.886
		Fort Rucker	.005	.149	1.000
		Suwannee	-.016	.149	1.000
		Ossabaw	.205	.149	1.000
		Patuxent	.017	.149	1.000
	Fort Rucker	Fort Bragg	.278	.148	.934
		Fort Campbell	-.005	.149	1.000
		Suwannee	-.021	.148	1.000
		Ossabaw	.200	.148	1.000
		Patuxent	.012	.148	1.000
	Suwannee	Fort Bragg	.299	.148	.676
		Fort Campbell	.016	.149	1.000
		Fort Rucker	.021	.148	1.000
		Ossabaw	.221	.148	1.000
		Patuxent	.033	.148	1.000
	Ossabaw	Fort Bragg	.078	.148	1.000
		Fort Campbell	-.205	.149	1.000
		Fort Rucker	-.200	.148	1.000
		Suwannee	-.221	.148	1.000
		Patuxent	-.188	.148	1.000
	Patuxent	Fort Bragg	.266	.148	1.000
		Fort Campbell	-.017	.149	1.000
		Fort Rucker	-.012	.148	1.000
		Suwannee	-.033	.148	1.000
		Ossabaw	.188	.148	1.000
flag6len	Fort Bragg	Fort Campbell	-1.545	1.639	1.000
		Fort Rucker	-3.622	1.639	.421
		Suwannee	-5.569(*)	1.628	.011
		Ossabaw	4.781	1.628	.055
		Patuxent	-.844	1.628	1.000
	Fort Campbell	Fort Bragg	1.545	1.639	1.000
		Fort Rucker	-2.077	1.649	1.000
		Suwannee	-4.024	1.639	.222
		Ossabaw	6.326(*)	1.639	.002
		Patuxent	.701	1.639	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Fort Rucker	Fort Bragg	3.622	1.639	.421
		Fort Campbell	2.077	1.649	1.000
		Suwannee	-1.947	1.639	1.000
		Ossabaw	8.403(*)	1.639	9.251E-06
		Patuxent	2.778	1.639	1.000
	Suwannee	Fort Bragg	5.569(*)	1.628	.011
		Fort Campbell	4.024	1.639	.222
		Fort Rucker	1.947	1.639	1.000
		Ossabaw	10.350(*)	1.628	1.621E-08
		Patuxent	4.725	1.628	.061
	Ossabaw	Fort Bragg	-4.781	1.628	.055
		Fort Campbell	-6.326(*)	1.639	.002
		Fort Rucker	-8.403(*)	1.639	9.251E-06
		Suwannee	-10.350(*)	1.628	1.621E-08
		Patuxent	-5.625(*)	1.628	.010
	Patuxent	Fort Bragg	.844	1.628	1.000
		Fort Campbell	-.701	1.639	1.000
		Fort Rucker	-2.778	1.639	1.000
		Suwannee	-4.725	1.628	.061
		Ossabaw	5.625(*)	1.628	.010
flag6wid	Fort Bragg	Fort Campbell	1.351	.480	.080
		Fort Rucker	.563	.477	1.000
		Suwannee	.900	.477	.910
		Ossabaw	1.238	.477	.152
		Patuxent	1.856(*)	.477	.002
	Fort Campbell	Fort Bragg	-1.351	.480	.080
		Fort Rucker	-.789	.480	1.000
		Suwannee	-.451	.480	1.000
		Ossabaw	-.114	.480	1.000
		Patuxent	.505	.480	1.000
	Fort Rucker	Fort Bragg	-.563	.477	1.000
		Fort Campbell	.789	.480	1.000
		Suwannee	.337	.477	1.000
		Ossabaw	.675	.477	1.000
		Patuxent	1.294	.477	.108
	Suwannee	Fort Bragg	-.900	.477	.910
		Fort Campbell	.451	.480	1.000
		Fort Rucker	-.337	.477	1.000
		Ossabaw	.338	.477	1.000
		Patuxent	.956	.477	.695
	Ossabaw	Fort Bragg	-1.238	.477	.152
		Fort Campbell	.114	.480	1.000
		Fort Rucker	-.675	.477	1.000
		Suwannee	-.338	.477	1.000
		Patuxent	.619	.477	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Patuxent	Fort Bragg	-1.856(*)	.477	.002
		Fort Campbell	-.505	.480	1.000
		Fort Rucker	-1.294	.477	.108
		Suwannee	-.956	.477	.695
		Ossabaw	-.619	.477	1.000
flag6rat	Fort Bragg	Fort Campbell	-.452	.160	.078
		Fort Rucker	-.378	.160	.286
		Suwannee	-.558(*)	.159	.008
		Ossabaw	-.124	.159	1.000
		Patuxent	-.607(*)	.159	.003
	Fort Campbell	Fort Bragg	.452	.160	.078
		Fort Rucker	.074	.161	1.000
		Suwannee	-.107	.160	1.000
		Ossabaw	.328	.160	.627
		Patuxent	-.156	.160	1.000
	Fort Rucker	Fort Bragg	.378	.160	.286
		Fort Campbell	-.074	.161	1.000
		Suwannee	-.181	.160	1.000
		Ossabaw	.254	.160	1.000
		Patuxent	-.229	.160	1.000
	Suwannee	Fort Bragg	.558(*)	.159	.008
		Fort Campbell	.107	.160	1.000
		Fort Rucker	.181	.160	1.000
		Ossabaw	.435	.159	.102
		Patuxent	-.049	.159	1.000
	Ossabaw	Fort Bragg	.124	.159	1.000
		Fort Campbell	-.328	.160	.627
		Fort Rucker	-.254	.160	1.000
		Suwannee	-.435	.159	.102
		Patuxent	-.483(*)	.159	.040
	Patuxent	Fort Bragg	.607(*)	.159	.003
		Fort Campbell	.156	.160	1.000
		Fort Rucker	.229	.160	1.000
		Suwannee	.049	.159	1.000
		Ossabaw	.483(*)	.159	.040
flag7len	Fort Bragg	Fort Campbell	-.902	1.589	1.000
		Fort Rucker	-3.150	1.632	.823
		Suwannee	-6.493(*)	1.632	.001
		Ossabaw	2.778	1.610	1.000
		Patuxent	-1.722	1.610	1.000
	Fort Campbell	Fort Bragg	.902	1.589	1.000
		Fort Rucker	-2.248	1.589	1.000
		Suwannee	-5.591(*)	1.589	.008
		Ossabaw	3.680	1.567	.296
		Patuxent	-.820	1.567	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Fort Rucker	Fort Bragg	3.150	1.632	.823
		Fort Campbell	2.248	1.589	1.000
		Suwannee	-3.343	1.632	.626
		Ossabaw	5.928(*)	1.610	.004
		Patuxent	1.428	1.610	1.000
	Suwannee	Fort Bragg	6.493(*)	1.632	.001
		Fort Campbell	5.591(*)	1.589	.008
		Fort Rucker	3.343	1.632	.626
		Ossabaw	9.271(*)	1.610	4.398E-07
		Patuxent	4.771	1.610	.051
	Ossabaw	Fort Bragg	-2.778	1.610	1.000
		Fort Campbell	-3.680	1.567	.296
		Fort Rucker	-5.928(*)	1.610	.004
		Suwannee	-9.271(*)	1.610	4.398E-07
		Patuxent	-4.500	1.587	.075
	Patuxent	Fort Bragg	1.722	1.610	1.000
		Fort Campbell	.820	1.567	1.000
		Fort Rucker	-1.428	1.610	1.000
		Suwannee	-4.771	1.610	.051
		Ossabaw	4.500	1.587	.075
flag7wid	Fort Bragg	Fort Campbell	1.137	.488	.312
		Fort Rucker	1.029	.502	.622
		Suwannee	.964	.502	.838
		Ossabaw	1.086	.495	.439
		Patuxent	1.694(*)	.495	.011
	Fort Campbell	Fort Bragg	-1.137	.488	.312
		Fort Rucker	-.109	.488	1.000
		Suwannee	-.173	.488	1.000
		Ossabaw	-.051	.481	1.000
		Patuxent	.557	.481	1.000
	Fort Rucker	Fort Bragg	-1.029	.502	.622
		Fort Campbell	.109	.488	1.000
		Suwannee	-.064	.502	1.000
		Ossabaw	.057	.495	1.000
		Patuxent	.665	.495	1.000
	Suwannee	Fort Bragg	-.964	.502	.838
		Fort Campbell	.173	.488	1.000
		Fort Rucker	.064	.502	1.000
		Ossabaw	.122	.495	1.000
		Patuxent	.730	.495	1.000
	Ossabaw	Fort Bragg	-1.086	.495	.439
		Fort Campbell	.051	.481	1.000
		Fort Rucker	-.057	.495	1.000
		Suwannee	-.122	.495	1.000
		Patuxent	.608	.488	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Patuxent	Fort Bragg	-1.694(*)	.495	.011
		Fort Campbell	-.557	.481	1.000
		Fort Rucker	-.665	.495	1.000
		Suwannee	-.730	.495	1.000
		Ossabaw	-.608	.488	1.000
flag7rat	Fort Bragg	Fort Campbell	-.385	.165	.307
		Fort Rucker	-.500	.169	.052
		Suwannee	-.645(*)	.169	.003
		Ossabaw	-.209	.167	1.000
		Patuxent	-.626(*)	.167	.003
	Fort Campbell	Fort Bragg	.385	.165	.307
		Fort Rucker	-.115	.165	1.000
		Suwannee	-.260	.165	1.000
		Ossabaw	.176	.163	1.000
		Patuxent	-.241	.163	1.000
	Fort Rucker	Fort Bragg	.500	.169	.052
		Fort Campbell	.115	.165	1.000
		Suwannee	-.145	.169	1.000
		Ossabaw	.291	.167	1.000
		Patuxent	-.126	.167	1.000
	Suwannee	Fort Bragg	.645(*)	.169	.003
		Fort Campbell	.260	.165	1.000
		Fort Rucker	.145	.169	1.000
		Ossabaw	.436	.167	.146
		Patuxent	.019	.167	1.000
	Ossabaw	Fort Bragg	.209	.167	1.000
		Fort Campbell	-.176	.163	1.000
		Fort Rucker	-.291	.167	1.000
		Suwannee	-.436	.167	.146
		Patuxent	-.417	.165	.182
	Patuxent	Fort Bragg	.626(*)	.167	.003
		Fort Campbell	.241	.163	1.000
		Fort Rucker	.126	.167	1.000
		Suwannee	-.019	.167	1.000
		Ossabaw	.417	.165	.182
r5l	Fort Bragg	Fort Campbell	24.142	17.808	1.000
		Fort Rucker	-17.050	17.578	1.000
		Suwannee	45.326	17.691	.166
		Ossabaw	74.659(*)	17.691	.001
		Patuxent	-20.900	17.578	1.000
	Fort Campbell	Fort Bragg	-24.142	17.808	1.000
		Fort Rucker	-41.192	17.808	.324
		Suwannee	21.184	17.919	1.000
		Ossabaw	50.517	17.919	.079
		Patuxent	-45.042	17.808	.181

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Fort Rucker	Fort Bragg	17.050	17.578	1.000
		Fort Campbell	41.192	17.808	.324
		Suwannee	62.376(*)	17.691	.008
		Ossabaw	91.709(*)	17.691	7.127E-06
		Patuxent	-3.850	17.578	1.000
	Suwannee	Fort Bragg	-45.326	17.691	.166
		Fort Campbell	-21.184	17.919	1.000
		Fort Rucker	-62.376(*)	17.691	.008
		Ossabaw	29.333	17.802	1.000
		Patuxent	-66.226(*)	17.691	.003
	Ossabaw	Fort Bragg	-74.659(*)	17.691	.001
		Fort Campbell	-50.517	17.919	.079
		Fort Rucker	-91.709(*)	17.691	7.127E-06
		Suwannee	-29.333	17.802	1.000
		Patuxent	-95.559(*)	17.691	2.467E-06
	Patuxent	Fort Bragg	20.900	17.578	1.000
		Fort Campbell	45.042	17.808	.181
		Fort Rucker	3.850	17.578	1.000
		Suwannee	66.226(*)	17.691	.003
		Ossabaw	95.559(*)	17.691	2.467E-06
wingwid	Fort Bragg	Fort Campbell	5.793	9.725	1.000
		Fort Rucker	-33.429(*)	9.536	.008
		Suwannee	21.795	9.596	.361
		Ossabaw	31.571(*)	9.536	.016
		Patuxent	-14.615	9.596	1.000
	Fort Campbell	Fort Bragg	-5.793	9.725	1.000
		Fort Rucker	-39.223(*)	9.666	.001
		Suwannee	16.001	9.725	1.000
		Ossabaw	25.777	9.666	.123
		Patuxent	-20.409	9.725	.554
	Fort Rucker	Fort Bragg	33.429(*)	9.536	.008
		Fort Campbell	39.223(*)	9.666	.001
		Suwannee	55.224(*)	9.536	3.457E-07
		Ossabaw	65.000(*)	9.476	9.641E-10
		Patuxent	18.814	9.536	.746
	Suwannee	Fort Bragg	-21.795	9.596	.361
		Fort Campbell	-16.001	9.725	1.000
		Fort Rucker	-55.224(*)	9.536	3.457E-07
		Ossabaw	9.776	9.536	1.000
		Patuxent	-36.410(*)	9.596	.003
	Ossabaw	Fort Bragg	-31.571(*)	9.536	.016
		Fort Campbell	-25.777	9.666	.123
		Fort Rucker	-65.000(*)	9.476	9.641E-10
		Suwannee	-9.776	9.536	1.000
		Patuxent	-46.186(*)	9.536	3.538E-05

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Patuxent	Fort Bragg	14.615	9.596	1.000
		Fort Campbell	20.409	9.725	.554
		Fort Rucker	-18.814	9.536	.746
		Suwannee	36.410(*)	9.596	.003
		Ossabaw	46.186(*)	9.536	3.538E-05
alpha	Fort Bragg	Fort Campbell	20.500	12.667	1.000
		Fort Rucker	-25.000	12.504	.701
		Suwannee	9.711	12.667	1.000
		Ossabaw	39.750(*)	12.504	.025
		Patuxent	-.250	12.504	1.000
	Fort Campbell	Fort Bragg	-20.500	12.667	1.000
		Fort Rucker	-45.500(*)	12.667	.006
		Suwannee	-10.789	12.829	1.000
		Ossabaw	19.250	12.667	1.000
		Patuxent	-20.750	12.667	1.000
	Fort Rucker	Fort Bragg	25.000	12.504	.701
		Fort Campbell	45.500(*)	12.667	.006
		Suwannee	34.711	12.667	.099
		Ossabaw	64.750(*)	12.504	7.325E-06
		Patuxent	24.750	12.504	.735
	Suwannee	Fort Bragg	-9.711	12.667	1.000
		Fort Campbell	10.789	12.829	1.000
		Fort Rucker	-34.711	12.667	.099
		Ossabaw	30.039	12.667	.278
		Patuxent	-9.961	12.667	1.000
	Ossabaw	Fort Bragg	-39.750(*)	12.504	.025
		Fort Campbell	-19.250	12.667	1.000
		Fort Rucker	-64.750(*)	12.504	7.325E-06
		Suwannee	-30.039	12.667	.278
		Patuxent	-40.000(*)	12.504	.024
	Patuxent	Fort Bragg	.250	12.504	1.000
		Fort Campbell	20.750	12.667	1.000
		Fort Rucker	-24.750	12.504	.735
		Suwannee	9.961	12.667	1.000
		Ossabaw	40.000(*)	12.504	.024
delta	Fort Bragg	Fort Campbell	-.618	10.411	1.000
		Fort Rucker	-9.250	10.277	1.000
		Suwannee	2.803	10.411	1.000
		Ossabaw	15.000	10.277	1.000
		Patuxent	-2.500	10.277	1.000
	Fort Campbell	Fort Bragg	.618	10.411	1.000
		Fort Rucker	-8.632	10.411	1.000
		Suwannee	3.421	10.544	1.000
		Ossabaw	15.618	10.411	1.000
		Patuxent	-1.882	10.411	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Fort Rucker	Fort Bragg	9.250	10.277	1.000
		Fort Campbell	8.632	10.411	1.000
		Suwannee	12.053	10.411	1.000
		Ossabaw	24.250	10.277	.287
		Patuxent	6.750	10.277	1.000
	Suwannee	Fort Bragg	-2.803	10.411	1.000
		Fort Campbell	-3.421	10.544	1.000
		Fort Rucker	-12.053	10.411	1.000
		Ossabaw	12.197	10.411	1.000
		Patuxent	-5.303	10.411	1.000
	Ossabaw	Fort Bragg	-15.000	10.277	1.000
		Fort Campbell	-15.618	10.411	1.000
		Fort Rucker	-24.250	10.277	.287
		Suwannee	-12.197	10.411	1.000
		Patuxent	-17.500	10.277	1.000
	Patuxent	Fort Bragg	2.500	10.277	1.000
		Fort Campbell	1.882	10.411	1.000
		Fort Rucker	-6.750	10.277	1.000
		Suwannee	5.303	10.411	1.000
		Ossabaw	17.500	10.277	1.000
beta	Fort Bragg	Fort Campbell	-2.158	7.068	1.000
		Fort Rucker	-9.500	6.976	1.000
		Suwannee	8.821	7.021	1.000
		Ossabaw	.500	6.976	1.000
		Patuxent	-6.500	6.976	1.000
	Fort Campbell	Fort Bragg	2.158	7.068	1.000
		Fort Rucker	-7.342	7.068	1.000
		Suwannee	10.978	7.112	1.000
		Ossabaw	2.658	7.068	1.000
		Patuxent	-4.342	7.068	1.000
	Fort Rucker	Fort Bragg	9.500	6.976	1.000
		Fort Campbell	7.342	7.068	1.000
		Suwannee	18.321	7.021	.145
		Ossabaw	10.000	6.976	1.000
		Patuxent	3.000	6.976	1.000
	Suwannee	Fort Bragg	-8.821	7.021	1.000
		Fort Campbell	-10.978	7.112	1.000
		Fort Rucker	-18.321	7.021	.145
		Ossabaw	-8.321	7.021	1.000
		Patuxent	-15.321	7.021	.452
	Ossabaw	Fort Bragg	-.500	6.976	1.000
		Fort Campbell	-2.658	7.068	1.000
		Fort Rucker	-10.000	6.976	1.000
		Suwannee	8.321	7.021	1.000
		Patuxent	-7.000	6.976	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Patuxent	Fort Bragg	6.500	6.976	1.000
		Fort Campbell	4.342	7.068	1.000
		Fort Rucker	-3.000	6.976	1.000
		Suwannee	15.321	7.021	.452
		Ossabaw	7.000	6.976	1.000
gamma	Fort Bragg	Fort Campbell	6.921	7.781	1.000
		Fort Rucker	-12.750	7.680	1.000
		Suwannee	20.936	7.729	.109
		Ossabaw	15.250	7.680	.724
		Patuxent	-6.250	7.680	1.000
	Fort Campbell	Fort Bragg	-6.921	7.781	1.000
		Fort Rucker	-19.671	7.781	.182
		Suwannee	14.015	7.829	1.000
		Ossabaw	8.329	7.781	1.000
		Patuxent	-13.171	7.781	1.000
	Fort Rucker	Fort Bragg	12.750	7.680	1.000
		Fort Campbell	19.671	7.781	.182
		Suwannee	33.686(*)	7.729	.000
		Ossabaw	28.000(*)	7.680	.005
		Patuxent	6.500	7.680	1.000
	Suwannee	Fort Bragg	-20.936	7.729	.109
		Fort Campbell	-14.015	7.829	1.000
		Fort Rucker	-33.686(*)	7.729	.000
		Ossabaw	-5.686	7.729	1.000
		Patuxent	-27.186(*)	7.729	.008
	Ossabaw	Fort Bragg	-15.250	7.680	.724
		Fort Campbell	-8.329	7.781	1.000
		Fort Rucker	-28.000(*)	7.680	.005
		Suwannee	5.686	7.729	1.000
		Patuxent	-21.500	7.680	.083
	Patuxent	Fort Bragg	6.250	7.680	1.000
		Fort Campbell	13.171	7.781	1.000
		Fort Rucker	-6.500	7.680	1.000
		Suwannee	27.186(*)	7.729	.008
		Ossabaw	21.500	7.680	.083
femur	Fort Bragg	Fort Campbell	40.667(*)	12.311	.017
		Fort Rucker	45.424(*)	12.311	.004
		Suwannee	80.474(*)	12.229	5.155E-09
		Ossabaw	96.105(*)	12.229	2.642E-12
		Patuxent	71.982(*)	12.398	3.361E-07
	Fort Campbell	Fort Bragg	-40.667(*)	12.311	.017
		Fort Rucker	4.757	12.393	1.000
		Suwannee	39.807(*)	12.311	.021
		Ossabaw	55.438(*)	12.311	.000
		Patuxent	31.315	12.479	.192

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Fort Rucker	Fort Bragg	-45.424(*)	12.311	.004
		Fort Campbell	-4.757	12.393	1.000
		Suwannee	35.050	12.311	.073
		Ossabaw	50.681(*)	12.311	.001
		Patuxent	26.559	12.479	.517
	Suwannee	Fort Bragg	-80.474(*)	12.229	5.155E-09
		Fort Campbell	-39.807(*)	12.311	.021
		Fort Rucker	-35.050	12.311	.073
		Ossabaw	15.632	12.229	1.000
		Patuxent	-8.491	12.398	1.000
	Ossabaw	Fort Bragg	-96.105(*)	12.229	2.642E-12
		Fort Campbell	-55.438(*)	12.311	.000
		Fort Rucker	-50.681(*)	12.311	.001
		Suwannee	-15.632	12.229	1.000
		Patuxent	-24.123	12.398	.795
	Patuxent	Fort Bragg	-71.982(*)	12.398	3.361E-07
		Fort Campbell	-31.315	12.479	.192
		Fort Rucker	-26.559	12.479	.517
		Suwannee	8.491	12.398	1.000
		Ossabaw	24.123	12.398	.795
tibia	Fort Bragg	Fort Campbell	93.245(*)	27.679	.013
		Fort Rucker	6.769	27.312	1.000
		Suwannee	77.282	27.312	.076
		Ossabaw	155.424(*)	27.141	4.914E-07
		Patuxent	28.252	27.875	1.000
	Fort Campbell	Fort Bragg	-93.245(*)	27.679	.013
		Fort Rucker	-86.475(*)	27.679	.030
		Suwannee	-15.963	27.679	1.000
		Ossabaw	62.180	27.510	.371
		Patuxent	-64.992	28.235	.334
	Fort Rucker	Fort Bragg	-6.769	27.312	1.000
		Fort Campbell	86.475(*)	27.679	.030
		Suwannee	70.513	27.312	.157
		Ossabaw	148.655(*)	27.141	1.736E-06
		Patuxent	21.483	27.875	1.000
	Suwannee	Fort Bragg	-77.282	27.312	.076
		Fort Campbell	15.963	27.679	1.000
		Fort Rucker	-70.513	27.312	.157
		Ossabaw	78.142	27.141	.066
		Patuxent	-49.030	27.875	1.000
	Ossabaw	Fort Bragg	-155.424(*)	27.141	4.914E-07
		Fort Campbell	-62.180	27.510	.371
		Fort Rucker	-148.655(*)	27.141	1.736E-06
		Suwannee	-78.142	27.141	.066
		Patuxent	-127.172(*)	27.708	.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Patuxent	Fort Bragg	-28.252	27.875	1.000
		Fort Campbell	64.992	28.235	.334
		Fort Rucker	-21.483	27.875	1.000
		Suwannee	49.030	27.875	1.000
		Ossabaw	127.172(*)	27.708	.000
cercusl	Fort Bragg	Fort Campbell	6.216	4.255	1.000
		Fort Rucker	-9.462	4.281	.421
		Suwannee	3.404	4.255	1.000
		Ossabaw	-5.481	4.281	1.000
		Patuxent	-6.159	4.255	1.000
	Fort Campbell	Fort Bragg	-6.216	4.255	1.000
		Fort Rucker	-15.678(*)	4.255	.004
		Suwannee	-2.813	4.228	1.000
		Ossabaw	-11.697	4.255	.097
		Patuxent	-12.375	4.228	.056
	Fort Rucker	Fort Bragg	9.462	4.281	.421
		Fort Campbell	15.678(*)	4.255	.004
		Suwannee	12.865(*)	4.255	.042
		Ossabaw	3.981	4.281	1.000
		Patuxent	3.303	4.255	1.000
	Suwannee	Fort Bragg	-3.404	4.255	1.000
		Fort Campbell	2.813	4.228	1.000
		Fort Rucker	-12.865(*)	4.255	.042
		Ossabaw	-8.885	4.255	.568
		Patuxent	-9.563	4.228	.369
	Ossabaw	Fort Bragg	5.481	4.281	1.000
		Fort Campbell	11.697	4.255	.097
		Fort Rucker	-3.981	4.281	1.000
		Suwannee	8.885	4.255	.568
		Patuxent	-.678	4.255	1.000
	Patuxent	Fort Bragg	6.159	4.255	1.000
		Fort Campbell	12.375	4.228	.056
		Fort Rucker	-3.303	4.255	1.000
		Suwannee	9.563	4.228	.369
		Ossabaw	.678	4.255	1.000
sperml	Fort Bragg	Fort Campbell	6.796(*)	1.402	3.620E-05
		Fort Rucker	7.471(*)	1.486	1.582E-05
		Suwannee	10.225(*)	1.527	2.879E-09
		Ossabaw	9.115(*)	1.411	1.055E-08
		Patuxent	8.892(*)	1.420	3.132E-08
	Fort Campbell	Fort Bragg	-6.796(*)	1.402	3.620E-05
		Fort Rucker	.675	1.477	1.000
		Suwannee	3.429	1.519	.375
		Ossabaw	2.319	1.402	1.000
		Patuxent	2.096	1.411	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Fort Rucker	Fort Bragg	-7.471(*)	1.486	1.582E-05
		Fort Campbell	-.675	1.477	1.000
		Suwannee	2.754	1.597	1.000
		Ossabaw	1.644	1.486	1.000
		Patuxent	1.421	1.495	1.000
	Suwannee	Fort Bragg	-10.225(*)	1.527	2.879E-09
		Fort Campbell	-3.429	1.519	.375
		Fort Rucker	-2.754	1.597	1.000
		Ossabaw	-1.110	1.527	1.000
		Patuxent	-1.333	1.536	1.000
	Ossabaw	Fort Bragg	-9.115(*)	1.411	1.055E-08
		Fort Campbell	-2.319	1.402	1.000
		Fort Rucker	-1.644	1.486	1.000
		Suwannee	1.110	1.527	1.000
		Patuxent	-.223	1.420	1.000
	Patuxent	Fort Bragg	-8.892(*)	1.420	3.132E-08
		Fort Campbell	-2.096	1.411	1.000
		Fort Rucker	-1.421	1.495	1.000
		Suwannee	1.333	1.536	1.000
		Ossabaw	.223	1.420	1.000
spermw	Fort Bragg	Fort Campbell	.277	.730	1.000
		Fort Rucker	.671	.774	1.000
		Suwannee	1.146	.795	1.000
		Ossabaw	.808	.734	1.000
		Patuxent	-.528	.739	1.000
	Fort Campbell	Fort Bragg	-.277	.730	1.000
		Fort Rucker	.394	.769	1.000
		Suwannee	.869	.791	1.000
		Ossabaw	.531	.730	1.000
		Patuxent	-.805	.735	1.000
	Fort Rucker	Fort Bragg	-.671	.774	1.000
		Fort Campbell	-.394	.769	1.000
		Suwannee	.475	.831	1.000
		Ossabaw	.137	.774	1.000
		Patuxent	-1.199	.778	1.000
	Suwannee	Fort Bragg	-1.146	.795	1.000
		Fort Campbell	-.869	.791	1.000
		Fort Rucker	-.475	.831	1.000
		Ossabaw	-.338	.795	1.000
		Patuxent	-1.674	.800	.562
	Ossabaw	Fort Bragg	-.808	.734	1.000
		Fort Campbell	-.531	.730	1.000
		Fort Rucker	-.137	.774	1.000
		Suwannee	.338	.795	1.000
		Patuxent	-1.336	.739	1.000

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
	Patuxent	Fort Bragg	.528	.739	1.000
		Fort Campbell	.805	.735	1.000
		Fort Rucker	1.199	.778	1.000
		Suwannee	1.674	.800	.562
		Ossabaw	1.336	.739	1.000
spermrat	Fort Bragg	Fort Campbell	.275	.099	.085
		Fort Rucker	.237	.104	.365
		Suwannee	.311	.107	.062
		Ossabaw	.296(*)	.099	.047
		Patuxent	.457(*)	.100	.000
	Fort Campbell	Fort Bragg	-.275	.099	.085
		Fort Rucker	-.039	.104	1.000
		Suwannee	.036	.107	1.000
		Ossabaw	.021	.099	1.000
		Patuxent	.182	.099	1.000
	Fort Rucker	Fort Bragg	-.237	.104	.365
		Fort Campbell	.039	.104	1.000
		Suwannee	.074	.112	1.000
		Ossabaw	.060	.104	1.000
		Patuxent	.220	.105	.555
	Suwannee	Fort Bragg	-.311	.107	.062
		Fort Campbell	-.036	.107	1.000
		Fort Rucker	-.074	.112	1.000
		Ossabaw	-.015	.107	1.000
		Patuxent	.146	.108	1.000
	Ossabaw	Fort Bragg	-.296(*)	.099	.047
		Fort Campbell	-.021	.099	1.000
		Fort Rucker	-.060	.104	1.000
		Suwannee	.015	.107	1.000
		Patuxent	.161	.100	1.000
	Patuxent	Fort Bragg	-.457(*)	.100	.000
		Fort Campbell	-.182	.099	1.000
		Fort Rucker	-.220	.105	.555
		Suwannee	-.146	.108	1.000
		Ossabaw	-.161	.100	1.000

* The mean difference is significant at the .05 level.

Appendix E

Alignment of CO1 sequences

(1)

(111)

(221)

(331)

(441)

Appendix F

Alignment of ITS2 sequences


(1)

(111)

(221)