Inferring haplotype diversity and population structure of the mite *Spinturnix americanus* between two host species, *M. lucifugus* and *M. septentrionalis*, via cytochrome b sequencing

by

Amanda Brown

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Abstract

The wing mite *Spinturnix americanus* is a sanguivorous and obligate ectoparasite on bats of the genus *Myotis*, including *Myotis lucifugus* (little brown bat) and *Myotis septentrionalis* (northern long-eared bat). These hosts have different social structures and life histories with respect to roost group size, travel distances between roosts and hibernacula, roost social behavior, foraging behavior, and specialist versus generalist roost tendencies. Genetic analyses conducted on similar European *Spinturnix* species found that these host life history traits influence the genetic diversity and genetic structure of their parasite populations. Using samples collected from *M. lucifugus* and *M. septentrionalis* hosts captured at the entrance of Hayes cave (Nova Scotia) during swarming in August-September 2006. I sequenced *cytochrome-b* and estimated genetic diversity (gene diversity and nucleotide diversity) and genetic differentiation (F_{ST}, and Φ_{ST}) levels in parasites collected from both host species. Twelve haplotypes were characterized from 28 individuals (20 from *S. americanus* mites collected from *M. lucifugus*, and 8 collected from *M. septentrionalis*), with 11 present in the *M. lucifugus* group, and 2 in *M. septentrionalis* group. One haplotype was found in 15 (54%) of the mites, (8 collected from *M. lucifugus*, 7 from *M. septentrionalis*). Estimates of gene diversity were 0.8421 and 0.2500, and nucleotide diversity were 0.0056 and 0.0023, for mites on *M. lucifugus* and *M. septentrionalis* respectively, indicating greater haplotype diversity in mites from *M. lucifugus*. F_{ST} and Φ_{ST} values were 0.1059 ($p= 0.0498$), and -0.0235 ($p= 0.6353$) suggesting some degree, but not uninhibited, gene flow between the parasites on each species, leading to some degree of genetic differentiation. The larger F_{ST} value compared to Φ_{ST} suggests that the movement rate is higher than the mutation rate, with mites spreading new mutations (haplotypes) among host species. The measures of genetic diversity suggest that *M. lucifugus'* life history factors of larger roost groups, longer roost to hibernacula migration distances, and more generalist roosting tendencies may facilitate more opportunity for mite interbreeding, and therefore greater genetic diversity. Future studies should be conducted to determine definitive degree of differentiation between the host groups, and to assess if they qualify as subpopulations with minimal or no gene flow between them, and observe how population dynamics have changed after the white-nose syndrome epidemic.

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Introduction

1.1 *Parasites*

Many mammalian species serve as hosts to ectoparasites. Among these animals are various bat species, which are frequently parasitized by a wide range of ectoparasites that cause varying degrees of cost to the host. Some parasites may cause no detriment to the bat host; whereas others my result in energetic costs of grooming and subsequent decreases in fitness, or serve as vectors for various pathogens, which can result in increased mortality (Lourenço and Palmeirim, 2007, Ritzi and Whitaker 2003). These ectoparasites range from obligate parasites to insects that sporadically feed on the host, and include chiggers, bat bugs, fleas and wing mites (Czenze and Broders 2011, Reindhardt and Siva-Jothy 2006, Rudnick 1960, Dick et al. 2003). Not only can ectoparasites have a profound effect on the fitness of their bat hosts, but studies conducted on various bat ectoparasites (particularly obligate wing mites of the genus *Spinturnix*) have shown that the reverse can be true as well. Various life history factors of the host bats can influence the likelihood of mite transfer among hosts and therefore mating dynamics, influencing levels of population structure and genetic diversity, which can impact the evolutionary trajectory of the parasite species and capacity to adapt in the face of potential local extinctions (Bruyndockx et al 2009, Mccoy 2009, Schaik et al.2015).

Myotis lucifugus (little brown bats) and *Myotis septentrionalis* (northern long-eared bat) are the two main species of bats found in Atlantic Canada, and are parasitized by many taxa of ectoparasites, although the degree of influence host life history factors have on the population

structure and genetic diversity of any of their ectoparasite species remains to be explored (Fenton and Barclay 1980, Mullen and Durden 2002, Smith and Clay 1988, Olson et al. 1978, Dick et al. 2003). *Myotis lucifugus* and *M. septentrionalis* are predominantly parasitized by the chigger species *Euchongastia pipistrelle*, *Euchongastia hamitoni*, and *Leptotrombidium myoti,* the flea species *Myodopsylla insignis*, the bat bug *Cimex adjunctus*, and the wing mite *Spinturnix americanus* (Entomological Society of Manitoba 2002, Fenton and Barclay 1980, Brennan 1947, Ebeling 1975, Mullen and Durden 2002).

Unlike chiggers and bat fleas, which are either entirely free-living or have at least one free-living stage in their life cycle, wing mites of the family *Spinturnicidae* are exclusively parasitic on bats, without a free-living stage in their life cycle (Rudnick 1960, Dick et al. 2003). Mites in laboratory settings were only able to survive a maximum of 2 days upon being separated from a host (Rudnick 1960).

Spinturnix americanus, the predominate Spinturnix mite found in Atlantic Canada, has a geographic range across Nearctic and neotropical America (Dick et al. 2003). The habitat of *Spinturnix americanus* is the wing membrane. *S. americanus* has several morphological adaptations that facilitate continuous wing habitation such as thick legs with heavy curved claws, no independent larval stage, and a requirement for a continuous diet of blood (Rudnick 1960, Poissant and Broders 2008).

The life cycle of *S. americanus* has 5 major stages: egg, larva, protonymph, deutonymph, and adult (Rudnick 1960). The egg and larval stages occur within the pregnant female mite, who gives birth to the protonymph directly, which then molts once into a male or female deutonymph (which is larger, has a narrower setal plate, and more setae), and finally molts once more into the sexually mature adult male or female mite which is the largest morph, with the full number of setae (Morales-Malacara and Lopez 1998, Rudnick 1960). Much like bat fleas, *S. americanus* appears to parasitize females more frequently than males, possibly because the colonial social structure among female bats facilitates mite population growth (Poissant and Broders 2008, Bruyndonckx et al. 2010).

1.2 *Ecology with host species*

Compared to other common bat parasites, wing mites of the *Spinturnix* genus, due to their status as obligate parasites, are optimal for studying how the life history and ecology hosts impacts parasite genetics (Bruyndockx et al 2009, Mccoy 2009, Schaik et al.2015). Being obligate parasites means wing-mite mating, population, and interbreeding dynamics are, in many ways, dependent on host ecological factors. This influence on mite mating and interbreeding patterns in turn can shape the genetic makeup of the population (or sub-population) in terms of genetic diversity and genetic structure (Bruyndockx et al 2010). The host life history factors that can potentially influence wing mite mating dynamics-and therefore genetic diversity and population structure-are host foraging patterns, roost size, roost fidelity, migration distance between summer roosts and winter hibernacula, roost/hibernacula type (man-made versus natural), mating behavior, and colony social systems (Bruyndockx et al 2009, Mccoy 2009, Schaik et al.2015).

Previous studies on the European *Spinturnix* species, *S. bechsteinii*, and *S. myotis* (whose bat hosts are *M. bechsteinii* and *M. myotis*, respectively), found that the host ecological factors of increased migration and travel distances and larger roost sizes were associated with increased genetic diversity and weaker population structure for *Spinturnix* mites (Bruyndockx et al 2009, Mccoy 2009, Schaik et al.2015). Individuals of *S. myotis*' host species, *Myotis myotis,* visit other bat maternity colonies (of both the same and different species), form temporary harems with several females per roost, and travel over 50 km between summer and winter roosts (Bruyndonckx et al 2009). Conversely, individuals of *S. bechsteinii*'s host- *M. bechsteinii*- do not visit other maternity colonies, mate during the night without forming sustained swarming sites, travel less than 30 km between summer and winter, and form smaller hibernation clusters (Bruyndockx et al 2009, Mccoy 2009, Schaik et al.2015). Therefore, in *M. bechsteinii*, transmission of ectoparasites among individuals of different colonies can only occur during mating or hibernation (Bruyndonckx et al 2009).

Due to the increased inter and intra-colony contact of individuals in *M. myotis* compared to *M. bechsteinii, Spinturnix myoti* has more dispersal potential, resulting higher genetic diversity and lower pairwise differentiation among colonies sampled in Switzerland, France, and Spain (Schaik et al 2014, Bruyndonckx et al 2009). Forty-nine cytochrome-*b* haplotypes have been identified from 119 *Spinturnix myoti* mites, with 9 haplotypes being shared across colonies; compared to only 23 haplotypes from 402 sampled *S. bechsteini* mites with 3 haplotypes shared across colonies (Schaik et al. 2014, Bruyndonckx et al 2009). *Spinturnix bechsteini* has less

genetic diversity, and strongly differentiated population structure compared to *Spinturnix myoti* (Schaik et al. 2014, Bruyndonckx et al 2009).

Whether or not this relationship between host life history and parasite genetic diversity applies to the predominate wing mite species in Nova Scotia- *Spinturnix americanus*- has never previously been studied. However, since *S. americanus* frequently persists on two Atlantic *Myotis* species with varying life histories and social systems- *Myotis lucifugus* and *Myotis septentrionalis*- it would be an ideal species to use study this phenomenon.

Three main species of Atlantic Canadian bats are *Myotis lucifugus*, and *Myotis septentrionalis*, and the tri-colored Bat (*Perimyotis subflavus*), the last of which will not be represented in this study due to lack of mites collected from this species among the available samples (Farrow and Broders 2011, Carstens and Dewey 2010). Atlantic *Myotis* bats are found throughout much of North America, and are year-round residents in Atlantic Canada, with swarming and hibernation sites in Nova Scotia and New Brunswick (Farrow and Broders 2011). They are insectivorous microchiropteran with brown pelage (Furlonger et al. 1987). Atlantic *Myotis* bats are promiscuous species whose social structure consists of solitary males and females that form summer maternity roosts (Thomas et al. 1979, Wai-Ping and Fenton 1988). They hibernate throughout the winter months and swarm throughout autumn (Burns and Broders 2015, Thomas et al. 1979). While once abundant in the Atlantic Provinces, all of the *Myotis* species have seen a population reduction of at least 94% since 2010 due to the epidemic of white nose syndrome, caused by the fungus *Pseudogymnoascus destructans* (Dzal et al. 2010).

The little brown bat (*M. lucifugus*) typically weighs between 6-9g, with a length of 60-102 mm (Fenton and Barclay 1980). They are insectivorous and mainly forage over water (Belwood and Fenton 1976, Buchler 1976). They form large summer roosts (which can contain thousands of individuals), frequently in buildings and other man-made structures (Fenton and Barclay 1980, Davis and Hitchcock 1965). *Myotis lucifugus* bats often travel several hundred kilometers between summer roosts and hibernacula (with distances up to 650 km), and commonly show roost fidelity (although some roost switching behavior has been observed), and are more likely to remain in the same roosts all summer (Norquay et al 2013, Broders and Forbes 2004, Foster and Kurta 1999, Olson and Barclay 2013). However, during swarming, roost groups mix with each other, providing a possible avenue for mite transfer and interbreeding (Segers and Broders 2015, Johnson et al. 2015).

In contrast, *M. septentrionalis* (which typically weighs of 5-8g, and an average length of 860 mm), is a forest specialist that roosts primarily in trees (Fenton and Barclay 1980, Foster and Kurta 1999). Roost group sizes tend to be smaller than in *M. lucifugus*, migration distances are typically under 300 km, and greater greater roost switching behavior with lower roost fidelity is observed (Foster and Kurta 1999, Johnson et al 2009). Maternity colonies in *M. septentrionalis* are also known for having a "fission-fusion" social system where associating individuals regularly move among multiple interconnected groups (although this may be true of *M. lucifugus* as well) (Garroway and Broders 2008).

These factors (such as roost sizes, travel distances, and roost fidelity and mixing) could create variant levels of genetic diversity in *S. americanus* mites based solely on host species ecology (Norquay et al 2013, Broders and Forbes 2004, Foster and Kurta 1999, Olson and Barclay 2013). That is, of course, as long as there is not uninhibited gene flow between mites on different hosts, allowing for all mites with variant haplotypes or genotypes to potentially interbreed, regardless of host species. These potential barriers to gene flow would result in population structure between mites from different hosts, which is likely because different host life history factors also create potential for mites on different hosts to have reproductive barriers to interbreeding (Schaik et al 2014, Bruyndonckx et al 2009, Schaik et al. 2015). Such factors include differences in roost selection and migration behavior, where bat hosts of different species are less likely to engage in close enough contact to facilitate mating in wing mites (Norquay et al 2013, Broders and Forbes 2004, Foster and Kurta 1999, Olson and Barclay 2013). In this case, there would be population structure between these mite groups, resulting in different levels of genetic diversity in the presence of variant host ecologies, which would create different gene pools (Schaik et al 2014, Bruyndonckx et al 2009, Schaik et al. 2015). As long as there is some degree of genetic differentiation present based on host species, variant levels of genetic diversity between the mites is possible, allowing host life history factors to influence the genetic diversity and potentially evolutionary trajectory of its parasitic mites.

1.3 *S. americanus and host influence on genetic diversity and population structure*

In this study, *S. americanus* mites collected from both *M. lucifugus* and *M. septentrionalis* will be sequenced and genetic diversity levels will be determined for each group to see if the life history and social structure differences between *M. lucifugus* and *M. septentrionalis* hosts result

in differing levels of genetic diversity for *S. americanus*. Degree of population structure and genetic differentiation will also be estimated to see if different haplotypes are accumulating between the groups. If genetic differentiation is present, then host species is a likely distinguishing line for two genetically distinct groups for *S. americanus.* These measures should ideally determine the degree of influence host life history factors have over the genetic diversity and structure of *S.americanus,* and indicate which host life history factors increase genetic diversity in the ectoparasites.

With these differences in social dynamics and life history in mind, I hypothesize that there will be little opportunity for interbreeding between mites on different host species, leading to moderate to high levels of genetic differentiation between mites on each host species. Because of this, different mutations and haplotypes should be accumulating independently in each group. I predict that mites on *M. lucifugus* will have higher levels of genetic diversity due to greater roost sizes and distances travelled between roosts and hibernacula, both of which are factors that facilitated greater genetic diversity in the European *Spinturnix* studies (Johnson et al. 2015, Segers and Broders 2015, Czenze and Broders 2011, Bruyndockx et al 2009, Mccoy 2009, Schaik et al.2015). However, the greater roost infidelity and day roost behavior and fission-fusion social structure in *M. septentrionalis* may create ample opportunity for across colony interbreeding for *S. americanus* mites with *M. septentrionalis* hosts, which could facilitate higher genetic diversity and the development of more novel haplotypes (Foster and Kurta 1999, Johnson et al 2009, Garroway and Broders 2008).

Methods

2.1 *Sample Selection*

Ectoparasite samples from *Myotis* bats in Atlantic Canada were previously collected by the Broders lab from 1999 to 2014, preserved in ethanol, and stored at -20^{o}C. First, species of the parasite samples were identified. Fleas, bat bugs, trombiculids, and spinturnids made up the bulk of the ectoparasite samples, and were identified via morphological means. Specifically, *Spinturnix* mites are arachnids with 4 pairs of legs, which are large in relation to the body. This is unlike chiggers, which have 3 pairs of legs during their parasitic phase, and have larger bodies compared to the legs. The characteristic feature of *S. americanus* is the presence of tiny setae on the posterdorsal sections of femorae III and IV, and the proximal sections of femorae I and II. Females of the species possess a rounded posterior of the idiosoma, and males have an idiosoma that narrows distally into a pointed opisthosoma section (Ebeling 1975, Rudnick 1960).

The *Spinturnix americanus* samples were further identified using the number and placement of setae, distinguishing it from the closely related species *S.bakeri*. In *S. bakeri*, the posteroventral setae on leg II and the anteroventral setae leg III are mostly long, the pair of proximal dorsal setae on femora I and II consist of one long and one short setae, and long proximal posterodorsal seta of femora III and IV (Ebeling 1975, Appendix A). In *S. americanus*, the ventral setae, and proximal dorsal setae of femora I and II are tiny compared to the other dorsal setae (Ebeling 1975, Rudnick 1960). There is also a difference in the number of dorsal opisthomal setae between *S.americanus* and *S. bakeri*; females of *S. americanus* typically present with 10-12, whereas males and females of *S. bakeri* present with only 4 (Shao et al 2006, Appendix A).

Other morphological characteristics of *S. americanus* include large dorsal shields, striated opisthomal integuments, small tritosternum, and a slightly long and posteriorly narrow epigynial shield (Shao et al 2006). *S. americanus* mites are typically around 1mm in length and width (Shao et al. 2005). *S. americanus* can be adequately identified and photographed with a dissecting or compound light microscope, as was done in this study. Once the parasites were identified, a parasite database was compiled so that research samples could be selected from among the mites that were the least degraded.

To control for genetic variation in the mites that may be due to male and female social structure differences in *Myotis* bats, spatial variation, and temporal variation, samples were selected entirely from female hosts, from only one site (Hayes Cave), and from within approximately a month sampling time (August 23rd – September 30th 2006). Forty-seven samples that met these criteria were selected for extraction and further analyses.

2.2 *DNA extraction*

First, the mites were transferred to 1.2 mL tubes, and crushed with a sterilized inoculating needle. Once crushed, 100 µl of lysis buffer (0.1M Tris, 4M Urea, 0.2M NaCl, 0.01M CDTA and 0.5% n-lauroylsarcosine) was added to the tubes. A positive control was also prepared using 0.05 g of calf thymus, and a negative control of lysis buffer was prepared as well. The samples were shaken periodically over 5 days to facilitate cell lysis. After 5 days, 10 µl of proteinase K was added to each tube, shaken, and left for 24 hours.

Following this, a second 10-µl spike of proteinase K was added to the samples. The samples were then placed in a 65ºC water-bath for 1 hour, then floated in a 37ºC incubator. A third and final addition of 10µl of proteinase K was added to the samples. After cell lysis, extraction was performed using a Qiagen DNeasy kit, according to the instructions.

2.3 *Cytochrome-b and Primers*

Cytochrome-*b* (cyt-*b*) was the site that was selected to be sequenced in this study, because it was the site used in the majority of the European studies on *Spinturnix* species, and has a wide range of universal primers that could potentially be used for arachnid species if a species- or genus-specific primer pair could not be found (Schaik et al 2014 , Bruyndonckx et al 2009, Schaik et al. 2011). The cytochrome-*b* is a region of the mitochondrial DNA (mtDNA) that is universal to eukaryotic cells and codes for the cytochrome-*b* protein; a component of the electron transport chain (Howell 1989, Espoti et al. 1993, Kocher et al.1989). This gene is frequently used in phylogenetic studies for the purpose of species discrimination, due to the fact that cyt-*b* -in many cases- is variable enough for species- and population-level discrimination, but conserved enough for the same primers to be used across a wide range of species (Castresana 2001, Bellis et al. 2003, Mccartney et al. 2003). Cytochrome-*b* sequencing can also be used to characterize population structure (via measures such as genetic differentiation between colonies and groups), and haplotype diversity (Bradley and Baker 2001, Garcia-Paris et al. 1999, Carr and Marshall 1991, Tanaka et al 1996). Since *S. americanus* has never previously been subject to genetic analysis, the versatility of many cyt-*b* primers makes this region an optimal selection for this

study (Meyer 1994). Selecting this gene for analysis also allows for direct comparisons to be more reasonably drawn between this study and related European studies, strengthening inferences about the effect of various life history factors on the genetic characteristics of parasite populations. Additionally, this ability to discriminate local level genetic differences between sub-populations and make inferences about distribution, genetic distance and diversity, and evolutionary trajectory within these genetically distinct groups makes cyt-*b* an ideal candidate for sequencing in this study (Helbig et al 1996).

The primer pair C1-J-2797mod and C1-J-2183 from one of the European *Spinturnix* studies was attempted to amplify *S. americanus*, but was not successful at annealing temperatures of 45ºC, 50ºC, and 55ºC, so the universal primer pair mcb 398 and mcb 869 as used instead, with success (Bruyndonckx et al 2009, Schaik et al. 2015).

2.4 *PCR Protocol and Sequencing Reactions*

Once the DNA was extracted, it was amplified using an experimentally determined PCR protocol. Two µl of the sample DNA was transferred to new tubes, with 18 µl of PCR cocktail (4.01 µl 1x PCR buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.4 µg/mL BSA, 0.3 µM of mcb 398 primer, 0.3 µM mcb 869 primer, 0.05 U/µl taq polymerase, and 6.71 µl deionized water). The PCR protocol used included a denaturation step for 1 cycle for 5 min. at 94ºC, then the annealing phase, which consists of 30 cycles (30 s at 94ºC, then 1 min. at 45ºC, and finally 1 min. at 72ºC), and finally an extension step of 45 min. at 60ºC. `

After PCR, an agarose gel was run to check approximate DNA concentration before the ExoSAP procedure was performed. Once this was accomplished, excess dNTPs were removed using the ExoSAP procedure (Dugan et al. 2002). For sequencing, we wanted 5-10ng of DNA for each 100 base pairs (bp) of desired sequence. I therefore standardized the PCR product of each sample to a concentration of 25-30 ng/µl and used 5 µl of this for subsequent analyses. An ExoSAP cocktail was mixed, consisting of Antarctic Phosphatase Buffer (0.65 µl per sample), Antarctic Phosphatase (0.1 µl per sample), Exonuclease I (0.03 µl per sample). Then a PCR program was run for 15 min at 37ºC, then 15 min at 80ºC, and held at 10ºC.

Next, the sequencing reaction was conducted, with a cocktail mix of 0.25X BigDye® Terminator reaction mix (1.5 µl per sample), 1X sequencing buffer (2.86 µl), 1 µl/rxn Primer (1 µl/rxn mcb 398), 5.78 µl/rxn mix, DNA (5.78 µl), and water (3.86 µl). PCR was performed with a 2 min. denaturing step at 94ºC, then a 30 cycle phase (20 s at 96ºC, then 20s at 50ºC, and finally 4 min at 60ºC), and finally held at 10ºC.

A de-salting protocol was then conducting prior to sequencing to remove salts from the samples prior to capillary electrophoresis (Irwin et al. 2003). First, 3.75 µl of 10 M ammonium acetate was added to each sample, 40 µl of 95% ethanol, which was mixed by pipetting up and down twice. Samples were then spun for 35 min at 2550 x g, the ethanol was then decanted, and the sample plate was spun inverted up to 300 rpm. After this, 100 µl of 70% ethanol was added to each sample and mixed with pipette. The sample plate was next spun for 2 minutes at 4550 x g,

and the ethanol was decanted gain, and inverted spinning up to 300 rpm. Next, the DNA was re-suspended in 10 µl of HiDi formamide in preparation for capillary electrophoresis.

After the de-salted samples were sequenced, 8 of the mite samples collected from *M. septentrionalis* hosts had sequences of poor quality, and were shown to have an excess concentration of DNA input into the ExoSAP reaction, and therefore subsequent sequencing reactions. ExoSAP was conducted a second time with the amplified products of these 8 samples, with 2 µl of product being added to the ExoSAP cocktail instead of the 5µl added to the other samples. All concentrations of other cocktail reagents were adjusted proportionally to obtain the same concentration of reagents as the previous sequencing reactions for ExoSAP, sequencing, and de-salting procedures.

2.5 *Sequence editing and haplotype analysis*

The de-salted samples were sequenced on and ABI 3500xl Genetic Analyser (Applied Biosystems), and the results were exported to 4Peaks to be clipped and edited for base pair clarity, before being converted to a fasta format. From there, the fasta files were loaded into ClustalX, and a haplotype chart was constructed based on the base pair differences present in the sequences after alignment.

From there, the haplotype chart was used as a reference to create an input file for Arlequin (Excoffier and Lischer 2015), which was used to estimate genetic differentiation and measures of

genetic diversity. In Arlequin, “Standard diversity indices” and “molecular diversity indices” were selected for computation, as well as standard AMOVA computations, using conventional F-statistics first (pairwise differences), and then using “compute distance matrix” (pairwise differences). This produced measures of F_{ST} (the proportion of variance in allele frequencies attributed to among population differences, compared to within population variance) and Φ_{ST} (a similar measure, specifically to quantify variance in haplotypic data), as well as measures of gene diversity, nucleotide diversity, and mean pairwise differences.

The Pegas package in R was then used to create a minimum spanning tree of the resulting haplotypes (Paradis).

Results

3.1 *Haplotypes*

After sequencing, 28 sequences had sufficient clarity for analyses (Table 1). The primers amplified a sequence of cyt-*b* consisting of 349 base pairs, 312 of which had enough clarity in enough samples to be considered 'useable' (Appendix C). The variable sites found were at positions 14, 15, 17, 41, 66, 136, 240, 276, 331, and 336, all consisting of 2 alleles, with the exception of locus 14 which contained 3 alleles (Table 2). Eight of these sequences were from mites collected from *M. septentrionalis* bats, and the remaining 20 were taken from *M. lucifugus* bats (Table 1).

Twelve haplotypes were found, 11 of which were found on mites from *M. lucifugus*, and two were from mites on *M. septentrionalis* (haplotypes 4 and 10). Haplotype 4 was the predominant haplotype with 15 of the 28 copies being this haplotype (8 *lucifugus*, 7 *M. septentrionalis*) (Table 1).

The constructed minimum spanning tree revealed that all haplotype sequences only differ by an increment of 1 bp (Figure 1). Haplotype 4, the most common haplotype, shared the most 1 bp difference connections with the other haplotypes at 5 (being varied by 1 bp with haplotypes 6, 7,2, and 12, and by 3 bp with haplotype 3) (Figure 1).

3.2 *Genetic Diversity Indices*

With respect to standard diversity indices, the *M. lucifugus* set had an estimated gene diversity of 0.8421 (SD +/- 0.0772), mean number of pairwise differences of 1.7526 (SD +/-1.0615), and an average nucleotide diversity of 0.0056 (SD +/- 0.0038) (Table 3). The corresponding values for the *M. septentrionalis* set were: gene diversity of 0.2500 (SD+/- 0.1802), mean number of pairwise differences of 0.7500 (SD +/- 0.6137), and an average nucleotide diversity of 0.0023 (SD+/- 0.0021) (Table 3).

The sources of haplotype diversity were substitution mutations (10 transitions, and 1 transversion), with no indels (Table 4). 10 transitions and the 1 transversion are present in the *M. lucifugus* set, and 3 transitions and no transversions were found in the *M. septentrionalis* set haplotypes (Table 4).

3.3 *Intra-host Differentiation*

The F_{ST} value comparing mites from the different host species was 0.1059 (p = 0.0498), and the Φ_{ST} estimate was -0.0235 (p= 0.6353) (Table 5, Table 6). In calculating F_{ST}, the percentage of variation among the two groups was 10.59%, versus 89.41% within the two groups. For Φ_{ST}, the percentage of variation among populations was 0.00%, and 100% within populations (Table 5, Table 6).

3.4 *Tables and Figures*

Table 1. Number of individual *S. americanus* mites with each cyt-*b* haplotype sequence, organized by bat host species**.** Collected from *M. lucifugus* and *M. septentrionalis* bats from Hayes cave NS August 23rd-September 30th 2006.

	Number of Individuals	
Haplotype Label	**M. lucifugus**	**M. septentrionalis**
1	1	0
2	1	0
3	1	0
4	8	7
5	2	0
6	1	0
7	1	0
8	1	0
9	2	0
10	0	1
11	1	0
12	1	0

Table 2. Polymorphic sites in the cyt-*b* sequence of *S. americanus*, arranged by haplotype.

	Variable Site									
Haplotype	**14**	**15**	**17**	**41**	**66**	**136**	**240**	**276**	**331**	**336**
1	A	G	T	A	C	C	T	A	G	C
2	A	G	T	A	C	C	T	A	G	T
3	A	G	T	A	C	T	T	A	A	T
4	A	G	T	G	C	C	T	A	G	T
5	A	G	T	G	C	C	T	A	G	C
6	A	G	T	G	C	C	T	G	G	T
7	A	G	C	G	C	C	T	A	G	T
8	A	A	T	G	C	C	T	G	G	T
9	G	G	T	G	C	C	T	A	G	C
10	G	G	T	G	C	C	C	A	G	C
11	G	G	T	G	T	C	T	A	G	C
12	T	G	T	G	C	C	T	A	G	T

Table 3. Genetic Diversity measures computed for cyt-*b* sequences of *S. americanus* arranged by host species.

	***lucifugus* host mites**	***septentrionalis* host mites**
Gene Diversity	0.8421(+/- 0.0772)	0.2500 (SD+/- 0.1802)
Mean # of pairwise differences	1.7526 (+/-1.0615)	0.7500 (SD+/- 0.6137)
Average nucleotide Diversity	0.0056 (+/- 0.0038)	0.0023 (SD+/- 0.0021)

Table 4. Molecular diversity indices for the cyt-*b* region of *S. americanus*.

Statistics	**M.lucifugus**	**M.septentrionalis**	**Total**
No. of transitions	9	3	11
No. of transversions	1	0	1
No. of substitutions	10	3	12
No. of indels	0	0	0
No. of transition sites	9	3	10
No. of transversion sites	1	0	1
No. of subst. sites	9	3	10
No. of indel sites	0	0	0

* Total does not equal *M.lucifugus* + *M.septentrionalis* numbers due to overlap of haplotypes between mites from both host groups.

Table 5. AMOVA results for Φ_{ST} Analysis of cyt-*b* of *S.americanus*, with *M. lucifugus* host mites and *M. septentrionalis* host mites treated as population groups.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	1	0.546	-0.01706 Va	0.00
Within populations	26	19.275	0.74135 Vb	100.00
Total	27	19.821	0.72429	
Fixation Index		**Φ_{ST} : -0.02355**		

Significance tests (1023 permutations)

Va and FST : P(rand. value > obs. value) = 0.59433
P(rand. value = obs. value) = 0.04106
P-value = 0.63539+-0.01284

Table 6. AMOVA results for F_{ST} Analysis of cyt-*b* of *S.americanus*, with *M. lucifugus* host mites and *M. septentrionalis* host mites treated as population groups.

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation
Among populations	1	0.804	0.04044 Va	10.59
Within populations	26	8.875	0.34135 Vb	89.41
Total	27	9.679	0.38179	
Fixation Index		**F_{ST} : 0.10593**		

Significance tests (1023 permutations)

Va and FST : P(rand. value > obs. value) = 0.03421

P(rand. value = obs. value) = 0.01564

P-value = 0.04985+-0.00572

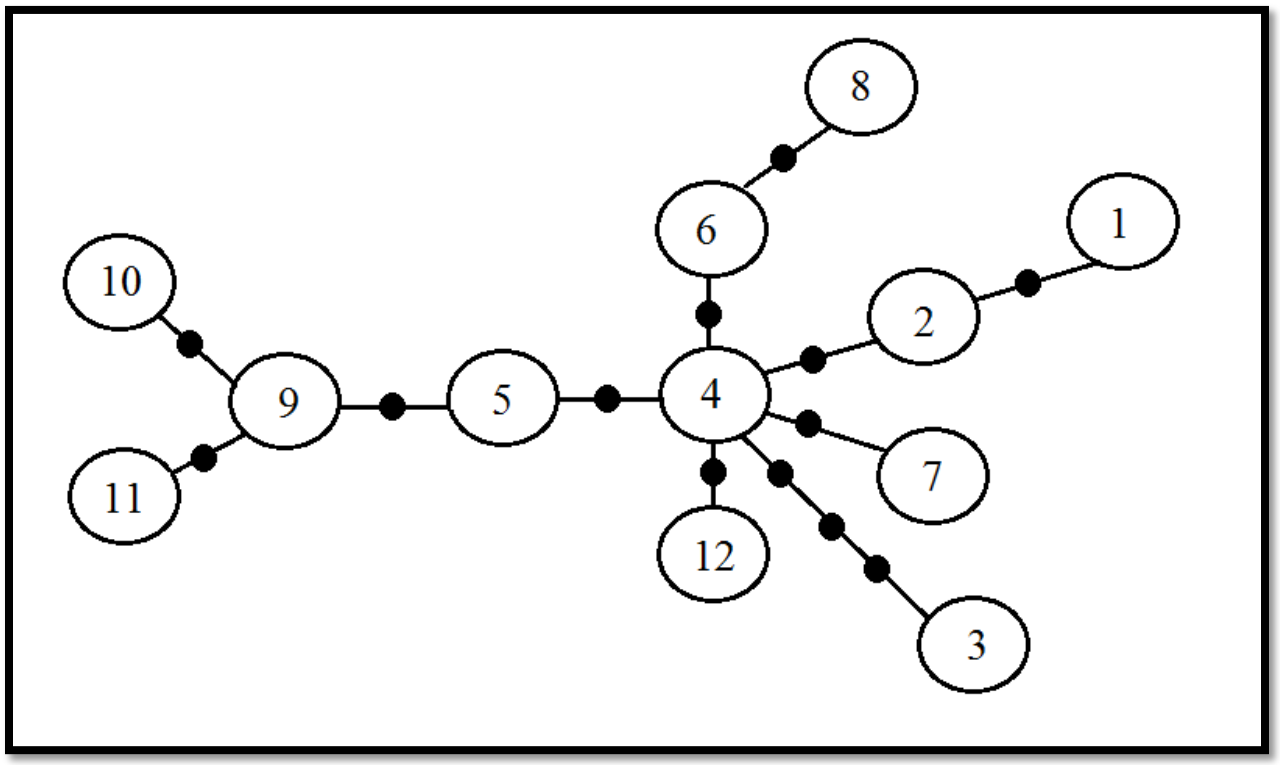

Fig 1. Minimum Spanning Tree of *S. americanus* haplotypes at the cyt-b region, by bp differences. Collected from *M. lucifugus* and *M. septentrionalis* bats from Hayes cave NS August 23rd-September 30th 2006**.**

Discussion

4.1 *Genetic Diversity*

Results indicate that mites on *M. lucifugus* did have higher genetic diversity, as predicted, compared to those on *M. septentrionalis*, but, while some degree of genetic differentiation appears to be present between the two groups, the estimates were not as high as predicted. Since only 8 mite samples collected from *M. septentrionalis* hosts had enough clarity in the amplified sequences to be analyzed in the study, the results may reflect the limitation in sample size, with more haplotypes potentially being present in this group, but not in the study samples. Regardless, the results demonstrate that *S. americanus* genetic diversity and population structure are likely influenced by host social dynamics to a certain degree, which has implications of potential ecological importance for the survival and evolutionary trajectory of parasite and host species alike.

Gene diversity, which in a haploid sample set is used as a measure of the probability that 2 randomly selected samples possess different haplotypes, was used as the primary measure to determine levels of haplotype diversity (Nei 1987). These results indicate that the *M. lucifugus* set possesses a larger number of haplotypes, and are therefore more genetically diverse than the mites from the *M. septentrionalis* set. This result is further substantiated by the respective levels of nucleotide diversity, where the value for the *M. lucifugus* data set was more than double that for *M. septentrionalis*. Both of these values are relatively low, which is expected due to the differing haplotypes varying by a maximum 7 nucleotides. Nucleotide diversity accounts for

degree of differences between haplotypes, not just whether or not they are different, so the results for this measure are expectedly lower than those for gene diversity (Nei 1987, Tajima 1983). Once again, the *M. lucifugus* set has a higher value, as expected. It also possessed a higher number of mean pairwise differences (1.7526 vs. 0.7500). The end result indicates higher nucleotide polymorphism and therefore suggests higher genetic diversity in the *M. lucifugus* set, as predicted.

4.2 *Host Life History Factors that Facilitate Parasite Genetic Diversity*

The sampling procedure attempted to control for geographic variability (all samples were collected from Hayes Cave site), temporal variability (all samples were collected in August-September 2006), and variability in social sexual dimorphism (all samples were collected from females). However, because samples were taken from a swarming site to ensure an adequate number of mites from both hosts present in one location, some geographic variability may be present because multiple roosts are represented at swarming site. However, in both host species, bats congregate at swarming sites from various roost sites, meaning roost geographic variability would be a factor in each host group, and should therefore not be responsible for differing genetic diversity levels between mites from each host group. Therefore, it can reasonably be concluded that the majority of differences in genetic diversity between *S. americanus* mites sequenced in this study is directly connected to the ecology, social behavior, and life histories of their hosts. And, as the results of this study suggest, *M. lucifugus* has a life history that facilitates the development of more haplotypes and greater genetic diversity among its *S. americanus* ectoparasites.

This is the result that would be expected based on the results of the studies on European *Spinturnix* and *Myotis* species. In those studies, *M. myotis* mites surpassed *M. bechsteinii* mites in genetic diversity, with *S. myotis* having over two-times as many haplotypes as *S. bechsteinii* (Schaik et. al 2014). Much like *M. myotis, M. lucifugus* is the host that occupies larger roosts, travels farther distances between hibernacula and roosts, and has more generalist tendencies compared to *M. septentrionalis* (Schaik et al. 2014, Bruyndonckx et al. 2009, Hofstede and Fenton 2005). *M. septentrionalis* does possess the fission-fusion social system, and less roost fidelity, which could have resulted in increased genetic diversity (Garroway and Broders 2008, Foster and Kurta 1999, Johnson et al 2009). However, the results of this study suggest that these factors do not facilitate genetic diversity in *S. americanus* mites to the same degree as *M. lucifugus* life history and social structure.

Roost intermixing during swarming is most likely the main source of interbreeding between *S. americanus* individuals that parasitize *M. lucifugus* bats (Johnson et. al 2015). This contact between bats of different geographic groups allows for transfer of the wing mites between these individuals, allowing geographically distinct mites to interbreed and transfer alleles between these roost groups in events of gene flow, fostering genetic diversity among *M. lucifugus* parasitizing mites. The greater distances traveled by *M. lucifugus* between roosts and hibernacula compared to *M. septentrionalis,* combined with the roost swarming behavior, could result in casting a geographical "wide net" for interbreeding during swarming, increasing the range of potential haplotypes present at swarming sites and increasing degree of gene flow and genetic diversity (Foster and Kurta 1999, Olson and Barclay 2013).

Perhaps most importantly, the sheer numbers of bats in the *M. lucifugus* roosts also increases the statistical probability that more mutations will arise there (so long as there is a corresponding high number of mites present as well), resulting in an accumulation of more unique haplotypes, which will also be present in the swarming sites come mating season (Davis and Hitchcock 1965).

It is easy to extrapolate how over 11 different haplotypes came to emerge in the *M. lucifugus* set of mites due to these factors. But why didn't factors such as greater roost infidelity and the fission fusion social system create the same (or a similar) degree of genetic diversity in the *M. septentrionalis* set? Both species have been observed to partake in the roost switching behavior to some degree, so this factor alone would not result in mites from *M. septentrionalis* bats exceeding those from *M. lucifugus* bats in measures of genetic diversity when the other *M. lucifugus* life factors are considered (Foster and Kurta 1999, Olson and Barclay 2013).

Additionally, both species have also been observed to engage in roost mixing behavior during swarming, so it is unlikely that roost mixing behavior is the key factor that enables greater haplotype diversity in *S. americanus* mites found on *M. lucifugus* bats (Johnson et. al 2015, Moussy et. al 2012). MtDNA analysis on *M. lucifugus* and *M. septentrionalis* bats in Atlantic Canada have shown weaker population structure for both species among swarming sites compared to roost sites, suggesting multiple roost groups assemble at these swarming sites

(Johnson et. al 2012). Here, they can interact closely enough to facilitate mite transfer between individuals, and therefore interbreeding among mites.

In a study by Johnson et al. (2015), F_{ST} values for summer versus swarming sites for *M. lucifugus* were 0.09300 vs 0.05200, and 0.1170 vs. 0.0430 for *M. septentrionalis*. These results suggest that there is actually *more* gene flow and lower genetic differentiation and genetic structure among *M. septentrionalis* bats at swarming sites (F_{ST} =0.0430, compared to 0.0520 in *M. lucifugus*). This finding indicates that there may be *more* roost mixing occurring with *M. septentrionalis* bats during swarming, which effectively eliminates the possibility that roost mixing results in higher haplotype diversity in wing mites found on *M. lucifugus*. The Johnson (2015) study reveals that for *M. lucifugus*, roost genetic structure and differentiation is 1.8x higher than at swarming sites, but an even greater 2.7x higher for *M. septentrionalis* roosts compared to swarming sites (Johnson et. al 2015).

This figure is important, because it helps establish that the factors of roost size and migration travel distance/pattern as the most probable key life history differences among hosts that influence *S. americanus* genetic structure. The higher F_{ST} value *M. septentrionalis* bats present with at roost sites compared to *M. lucifugus*, shows greater genetic differentiation among roosts. This implies that there is less free gene flow and genetic diversity at the roost level *M. septentrionalis* versus *M. lucifugus*? Dependence of *M. septentrionalis* on forests for roost sites- a factor that is not limiting for *M. lucifugus*- can lead to a greater scarcity of potential roost sites

to switch to during episodes of roost infidelity, resulting in spatial and limitations for mixing and mite interbreeding (Fenton and Barclay 1980).

The implications of the Johnson study, as well as the genetic diversity values found in this study, indicate that larger roost sizes, generalist tendencies, and greater travel distances between roosts and hibernacula are the factors that facilitate greater haplotype diversity among wing mites with *M. lucifugus* hosts, which is in accordance with the original hypothesis of this study (Johnson et. al 2015, Ellstrand et. al 2015, Furlan et. al 2012). These events create increased opportunity for mite transfer and interbreeding between *S. americanus* individuals on *M. lucifugus* hosts compared to those on *M. septentrionalis* hosts (Fenton 1969, Burns et. al 2014).

4.3 *Genetic Differentiation*

The AMOVA analysis produced F_{ST} and Φ_{ST} values that varied widely. The Φ_{ST} value between the *M. lucifugus* and *M septentrionali*s mites, which is an extremely low negative value, can be interpreted as approximating zero, and a large p-value >0.5, indicates that it is not significantly different from zero (Bortolotto et. al 2011). However, this should not be definitively interpreted as there being free gene flow between the populations. The F_{ST} value between the *M. lucifugus* and *M septentrionali*s mites of is statistically significant, and indicates that about 11% of the variation observed can be accounted for as variation among populations (due to genetic differentiation), the rest (and the majority) of the variation seems to be accounted for by differences within populations. The fact that the estimates of F_{ST} and Φ_{ST} are not equivalent is not necessarily problematic, as differences in these two values can be used to make inferences on

the relative roles of mutation and migration in shaping observed patterns of differentiation (Kronholm et. al 2010, Geraghty et. al 2013). For example, if $F_{ST} < \Phi_{ST}$, then there are more mutations accumulating within groups than there are migrants facilitating gene flow between the groups, which share new mutations that arise. The opposite is true when $F_{ST} > \Phi_{ST}$ (Kronholm et. al 2010, Geraghty et. al 2013).

Based on the results of this study, as F_{ST} is larger than Φ_{ST}, it would appear that there is enough migration between the groups that most of the new mutations that arise are able to cross over into the other group. This could occur during swarming, as mixing behavior is common, and all samples regardless of host species were collected from the same hibernacula (Johnson et. al 2015). None the less, the significant F_{ST} value suggests that there is not complete gene flow between the groups, as would be expected considering the mites are obligate parasites on different host species with minimal direct interaction most of the year (Johnson et. al 2015, Fenton and Barclay 1980).

The European studies that served as inspiration for this one examined genetic differentiation between populations of two different but closely related species. *S. myotis* was found to have a low Φ_{ST} of 0.012 and an F_{ST} for an examined nuclear DNA site of 0.002-0.026 (Schaik et. al 2014). Whereas *S. bechsteinii* had a mean nucDNA F_{ST} of 0.228 (Schaik et. al 2014). Initially, it was predicted that higher levels of genetic differentiation would be found for this study based on the fact that F_{ST} in the European study for *S. bechsteinii* populations sharing the same host was as high as 0.228, and the samples from this study had the added barrier of different hosts (Schaik et.

al 2014). However, the *S. myotis* F_{ST} value was 0.002-0.026, and Φ_{ST} was 0.012, so the F_{ST} value found in this study is not particularly unusual even though it is somewhat lower than predicted (Schaik et. al 2014). It is possible that the *S. bechsteinii* measures of genetic differentiation were higher than this study's because they were examining geographic isolation as opposed to those imposed by host species differences.

This low degree of genetic differentiation and limited gene flow can none the less assist in accounting for why haplotypes 1, 2, 3, 5, 6, 7, 8, 9, 11, and 12 are not present in the *M. septentrionalis* set of mites. While this may appear to be a large amount of haplotypes to be absent considering the small amount of genetic structure that is suggested by F_{ST}, all of the haplotypes other than haplotype 4 are only present in a maximum of 2 individuals from the final samples. It would not be unreasonable for many haplotypes present in small frequencies to not be carried by migration to the *M. septentrionalis* group with any degree of population structure in effect.

The F_{ST} and Φ_{ST} results, in conjunction with the measures of genetic diversity, indicate a modest degree of genetic differentiation is present between the two groups. However, with the sample size and variance between the F_{ST} and Φ_{ST}, it is difficult to definitively ascribe the exact degree of differentiation.

4.4 *Implications of Differentiation and Population Structure*

Given these results, it is not certain whether or not the groups will have different evolutionary trajectories. However, if the measures of genetic diversity found are reflective of the population at large, and there is some degree of genetic structure between the groups, then mites found of *M. lucifugus* may have a fitness advantage over *M. septentrionalis* mites (Reed and Frankham 2003). More genetically diverse populations with more variation in alleles are typically more likely to possess alleles that are more adaptive to the current environment and changing environmental conditions (Reed and Frankham 2003). White Nose Syndrome has most likely already caused a shift in host population composition as well as abundance, which could place a selection pressure on the mite populations to adapt to changing conditions (Brennan 1947). In the European studies, *S. bechsteinii,* with higher population structure and lower genetic diversity, was more susceptible to local extinctions and bottlenecks (Schaik et. al 2014). After the epidemic of White Nose Syndrome, it is likewise possible that mites from *M. septentrionalis* hosts, with lower genetic diversity, have been more likely to experience local extinctions or bottlenecks.

4.5 *Avenues of Future Research*

S. americanus' close relative, *S. bakeri*, was not identified in the mite database, so was not amplified and sequenced in this study for genetic comparison. However, since universal primers were used in this study, the mcb 398 and mcb 869 primer set is likely to amplify cyt-*b* in *S. bakeri* as well, which could prove a useful avenue of future research to provide a genetic basis of species discrimination in these morphologically similar wing mites (Verma and Singh 2002). Another avenue of potential future research could be to develop a species-specific primer set for *S. americanus*. Now that the predominant haplotype for the species appears to have been sequenced, this undertaking would be less difficult, and would prove helpful in ensuring host and

experimenter DNA is not amplified alongside with that of the mites. Having an *S. americanus* primer set would also prove helpful for future studies as the primer sets used in European *Spinturnix* species had no success in amplifying cyt-*b* in *S. americanus* (Bruyndonckx et al 2009, Schaik et al. 2015).

Genetic diversity levels in the *M. lucifugus*-attached mites is additionally of interest to future research endeavors, as higher genetic diversity may have already saved them from local extinction. Pre-existing higher levels of genetic variation can assist in lowering the effect of bottlenecking in events where the population is drastically reduced (Willi et. al 2006).

The White Nose Syndrome epidemic is a prime example of a dramatic population reduction that could have caused a bottleneck in *Myotis* populations, and the obligate ectoparasites that depend on them for their survival, such as *S. americanus* (Dzal et al. 2010). The samples used in this study pre-date the epidemic, so it would be a fascinating avenue of future research to compare haplotypes frequencies found in both groups now compared to 2006, if it is possible to collect a substantial amount of samples. Particularly, would mites currently found on *M. lucifugus* hosts be more prolific than those on *M. septentrionalis* hosts? This could prove an interesting means of evaluating whether the perceived greater genetic diversity found in this study was reflective of the population at large in 2006, and whether this resulted in increased fitness in the face of an epidemic of the hosts, vastly changing the mite's environmental conditions as well. Additionally, upon examining how the haplotype frequencies have changed and if bottlenecking

has occurred, it would be interesting to note if haplotype 4 is still the clear predominant haplotype in both groups.

Geographic isolation and differentiation was not selected as a variable to observe in this study, and its effect on genetic differentiation between wing mites could serve as an interesting further avenue of study in *S.americanus*, especially if it produces more differentiation than host species differences. Although, that may be improbable due mixing at swarm sites and roost infidelity, and would depend on the breadth of geographic sites examined. Genetic diversity differences between mites on male and female hosts could also prove a potential avenue of future research in the question of how much host life history plays a role in determining parasite genetic diversity, as male and female *Myotis* bats have varied social structures, with males being more solitary outside of swarming (Kunz 1982, Carter and Feldhamer 2005). Predictably, this should result in lower genetic diversity in mites attached to male hosts.

Further research focused on this family of blood feeding mites may be of interest to those studying the health and fitness of their bat hosts as well. Even though *S. americanus* does not appear to be the direct cause of serious health risks to their hosts, they may play a role in the transmission of rabies, encephalitis, and other pathogens in non- sanguivorous bats as vectors (Rudnick 1960). Wing mites have also been found to be more abundant on hosts considered to have weaker immunity such as juveniles and reproductive females (Giorgi et al. 2001). These host choices could cause further damages to bat colonies, as *Myotis myotis* bats parasitized by Spinturnix myotis during the maternity period are noted to use more oxygen and lose more

weight (Schaik et al 2014, Giorgi et al. 2001). By understanding the genetic diversity levels in *S. americanus* populations, general level of adaptability to local extinction can be inferred, allowing researchers to better determine the mite's chances of survivorship on an already ailing bat population, and the overall cost to bat colonies due to these infestations.

Conclusion

For the first time, *S. americanus* has been genetically sequenced, if particularly if *S. bakeri* is able to be sequenced as well, a genetic means of species discrimination in these closely related *Spinturnix* mites may be possible.

In addition to successful amplification of the mite's mtDNA at the cytochrome-*b* site, differences in genetic diversity between mites found on *M. lucifugus* and *M. septentrionalis* hosts was found. It can reasonably be extrapolated that the larger roost sizes, greater migration distances, and greater generalist tendencies of *M. lucifugus* results in more opportunity for *S.americanus* mites to accumulate different haplotypes and interbreed. In this way, host life histories have a direct effect on the genetic makeup and potential future adaptive capacity of their obligate parasites.

Analyses of population structure suggest that while there is some degree of gene flow between the parasites on the two species, there is not complete exchange of haplotypes between the groups and some degree of genetic differentiation and therefore population structure is present. The ramifications this has on the evolutionary trajectories of each group has yet to be determined and could provide for an exciting and valuable avenue of future research, particularly in light of the White Nose Syndrome epidemic that has ravaged *Myotis* bats in Atlantic Canada, and presumably their obligate parasites such as *S. americanus* as well.

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Appendix A

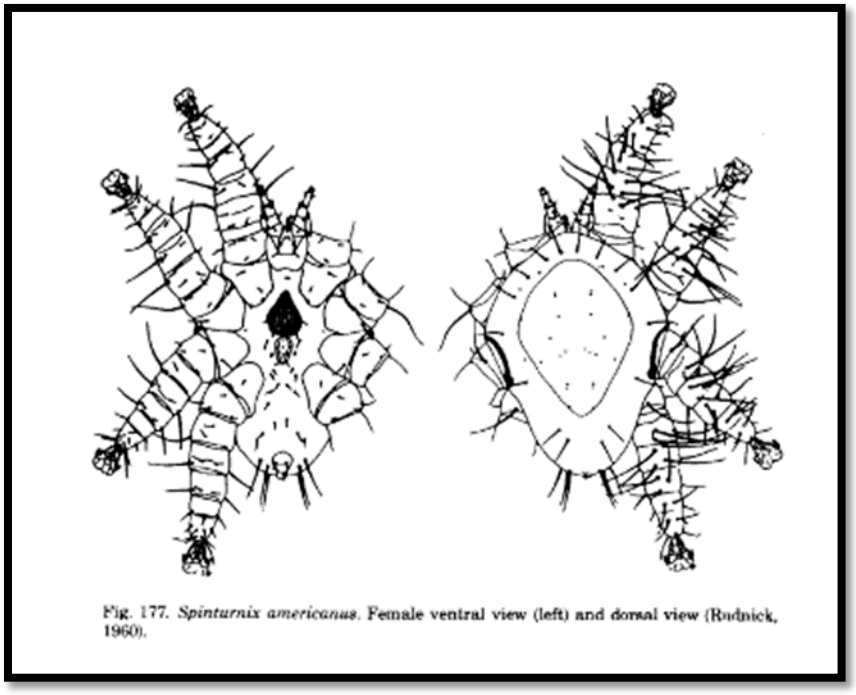

Fig. 177. *Spinturnix americanus*. Female ventral view (left) and dorsal view (Rudnick, 1960).

Figure 1. *Spinturnix americanus*, female ventral and dorsal view (from Rudnick 1960).

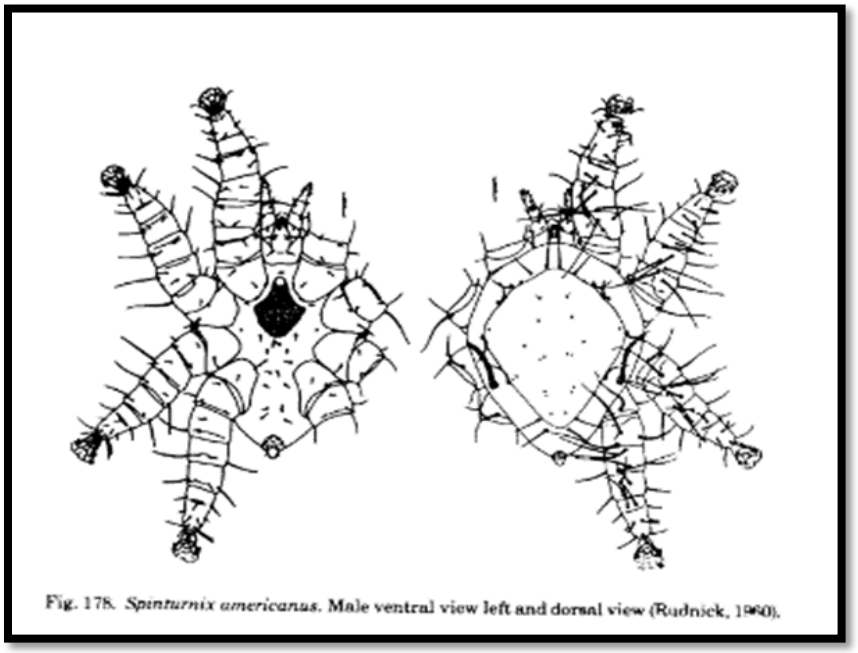

Fig. 178. *Spinturnix americanus*. Male ventral view left and dorsal view (Rudnick, 1960).

Figure 2. *Spinturnix americanus*, male ventral and dorsal view (from Rudnick 1960).

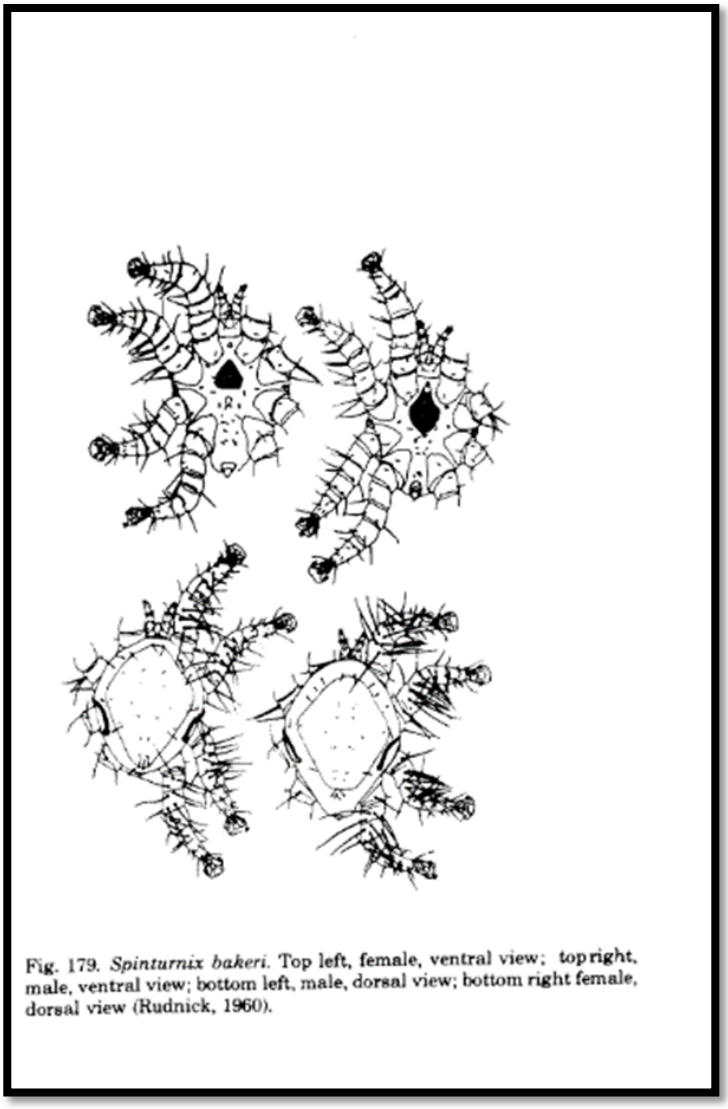

Fig. 179. *Spinturnix bakeri*. Top left, female, ventral view; top right, male, ventral view; bottom left, male, dorsal view; bottom right female, dorsal view (Rudnick, 1960).

Figure 3. *Spinturnix bakeri*, male (top right- ventral, bottom left-dorsal), and female (top left-ventral, bottom right-dorsal) ,from Rudnick (1960).

Appendix B

Table 1. *S. americanus* cytochrome-b haplotype list with corresponding sequences.

Haplotype 1	CCCATATATTGGAGATACACTTACATCTTGAATTTGAGGAGGNNNNNNNNNNAATAATGCCACA CTAACACGATTCTTTTCTTTANNNNNNNATTCTTCCTTTTATTCTAATAATATTTATTATAATTCA CATTCTTTTTCTTCATGAAACAGGCAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAA TTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAGTAGGTTATTTAATTTTTTTTATATTATTTAA TTATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAATTTTATTCCTGCTAATTCAATA GTAACCCCTATTCATATTC
Haplotype 2	CCCATATATTGGAGATACACTTACATCTTGAATTTGAGGAGGNNNNNNNNNNNNTAATGCCACA CTAACACGATTCTTTTCTTTANNNNNNNATTCTTCCTTTTATTCTAATAATATTTATTATAATTCA CATTCTTTTTCTTCATGAAACAGGCAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAA TTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAGTAGGTTATTTAATTTTTTTTATATTATTTAA TTATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAATTTTATTCCTGCTAATTCAATA GTAACTCCTATTCATATTC
Haplotype 3	CCCATATATTGGAGATACACTTACATCTTGAATTTGAGGAGGNNNNNNAATTAATAATGCCACA CTAACACGATTCTTTTCTTTACANTTTTATTCTTCCTTTTATTCTAATAATATTTATTATAATTCAC ATTTTTTTTCTTCATGAAACAGGCAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAAT TCCATTTCATCCTTTTTTTAGACTAAAGGATTTAGTAGGTTATTTAATTTTTTTTATATTATTTAAT TATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAATTTTATTCCTGCTAATTCAATAA TAACTCCTATTCATATTC
Haplotype 4*	CCCATATATTGGAGATACACTTACATCTTGAATTTGAGGGGGTNNNNNNNNNNNNAATGCCACA CTAACACGATTCTTTTCTTTACNNNNNNNNNNNNNCTTTTATTCTAATAATATTTATTATAATTCA CATTCTTTTTCTTCATGAAACAGGCAGAAGAAATCCTNNNNGTATCCCTTTAAACTTAGATAAAA TTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAGTAGGTTATTTAATTTTTTTTATATTATTTAA TTATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAATTTTATTCCTGCTAATTCAATA GTAACTCCTATTCATATTC
Haplotype 5	CCCATATATTGGAGATACACTTACATCTTGAATTTGAGGGGNNNNNNNNNNNAATAATGCCACA CTAACACGATTCTTTTCTTTANNNNNNTATTCTTCCTTTTATTCTAATAATATTTATTATAATTCAC ATTCTTTTTCTTCATGAAACAGGCAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAAT

	TCCATTTCATCCTTTTTTTAGACTAAAGGATTTAGTAGGTTATTTAATTTTTTTTATATTATTTAAT TATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAATTTTATTCCTGCTAATTCAATAG TAACCCCTATTCATATTC
Haplotype 6	CCCATATATTGGAGATACACTTACATCTTGAATTTGAGGGGGNNNNNNAATTAATAATGCCACA CTAACACGATTCTTTTCTTTACANTTTTATTCTTCCTTTTATTCTAATAATATTTATTATAATTCAC ATTCTTTTTCTTCATGAAACAGGCAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAAT TCCATTTCATCCTTTTTTTAGACTAAAGGATTTAGTAGGTTATTTAATTTTTTTTATATTATTTAAT TATATTATTTTGTTAAATCCTTTTATATTTTTTGATCCTGATAATTTTATTCCTGCTAATTCAATAG TAACTCCTATTCATATTC
Haplotype 7	CCCATATATTGGAGACACACTTACATCTTGAATTTGAGGGGGNNNNNNAATTAATAATGCCACA CTAACACGATTCTTTTCTTTACANTTTTATTCTTCCTTTTATTCTAATAATATTTATTATAATTCAC ATTCTTTTTCTTCATGAAACAGGCAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAAT TCCATTTCATCCTTTTTTTAGACTAAAGGATTTAGTAGGTTATTTAATTTTTTTTATATTATTTAAT TATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAATTTTATTCCTGCTAATTCAATAG TAACTCCTATTCATATTC
Haplotype 8	CCCATATATTGGAAATACACTTACATCTTGAATTTGAGGGGNNNNNNNNNNNNNTAATGCCACA CTAACACGATTCTTTTCTTTANNNNNNNNNNCTTCCTTTTATTCTAATAATATTTATTATAATTCA CATTCTTTTTCTTCATGAAACAGGCAGAAGAAATCCTTCAGGNATCCCTTTAAACTTAGATAAAA TTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAGTAGGTTNTTTAATTTTTTTTATATTATTTAA TTATATTATTTTGTTAAATCCTTTTATATTTTTTGATCCTGATAATTTTATTCCTGCTAATTCAATA GTAACTCCTATTCATATTC
Haplotype 9	CCCATATATTGGGGATACACTTACATCTTGAATTTGAGGGGGNNNNNNNNNNAATAATGCCACA CTAACACGATTCTTTTCTTTACANTTTTATTCTTCCTTTTATTCTAATAATATTTATTATAATTCAC ATTCTTTTTCTTCATGAAACAGGCAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAAT TCCATTTCATCCTTTTTTTAGACTAAAGGATTTAGTAGGTTATTTAATTTTTTTTATATTATTTAAT TATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAATTTTATTCCTGCTAATTCAATAG TAACCCCTATTCATATTC
Haplotype 10	CCCATATATTGGGGATACACTTACATCTTGAATTTGAGGGGGTTTNNNNNNTAATAATGCCACAC TAACACGATTCTTTTCTTTACANTTTTATTCTTCCTTTTATTCTAATAATATTTATTATAATTCACA TTCTTTTTCTTCATGAAACAGGCAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAATT CCATTTCATCCTTTTTTTAGACTAAAGGATTTAGTAGGTTACTTAATTTTTTTTATATTATTTAATT

	ATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAATTTTATTCCTGCTAATTCAATAGT AACCCCTATTCATATTC
Haplotype 11	CCCATATATTGGGGATACACTTACATCTTGAATTTGAGGGGNNNNNNNNNNNNNTAATGCCACA TTAACACGATTCTTTTCTTTANNNNNNNNTTCTTCCTTTTATTCTAATAATATTTATTATAATTCAC ATTCTTTTTCTTCATGAAACAGGCAGAAGAAATNNNNNAGGTATCCCTTTAAACTTAGATAAAAT TCCATTTCATCCTTTTTTTAGACTAAAGGATTTAGTAGGTTATTTAATTTTTTTTATATTATTTAAT TATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAATTTTATTCCTGCTAATTCAATAG TAACCCCTATTCATATTC
Haplotype 12	CCCATATATTGGTGATACACTTACATCTTGAATTTGAGGGGGNNNNNNNNTNAATAATGCCACA CTAACACGATTCTTTTCTTTACNNNNNNATTCTTCCTTTTATTCTAATAATATTTATTATAATTCAC ATTCTTTTTCTTCATGAAACAGGCAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAAT TCCATTTCATCCTTTTTTTAGACTAAAGGATTTAGTAGGTTATTTAATTTTTTTTATATTATTTAAT TATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAATTTTATTCCTGCTAATTCAATAG TAACTCCTATTCATATTC

*Predominant haplotype

Appendix C

Table 1. Computations for *S.americanus* data, output from Arlequin (Winarl35).

```
Project information:
--------------------
        NbSamples      =  2
        DataType       =  DNA
        GenotypicData  =  0




==============================
Settings used for Calculations
==============================


 General settings:
 -----------------
        Deletion Weight                = 1
        Transition Weight Weight       = 1
        Tranversion Weight Weight      = 1
        Epsilon Value                  = 1e-007
        Significant digits for output  = 5
        Use original haplotype definition
        Alllowed level of missing data = 0.05

 Active Tasks:
 -------------

    Standard indices:
    -----------------

    Molecular Diversity:
    --------------------
        Molecular Distance :Pairwise difference
        GammaA Value       = 0
        Theta estimators   :
            Theta(Hom)
            Theta(S)
            Theta(k)
            Theta(Pi)

        Compute minimum spanning network between haplotypes
        Print out inter-haplotypic distance matrix
        Compute Site Frequency Spectrum within populations
    Analysis of Molecular Variance:
    -------------------------------
        No. of Permutations = 1000
        Compute minimum spanning network between all haplotypes in the sample

       Distance matrix:
            Compute distance matrix
```

```
            Molecular distance : Pairwise difference
            Gamma a value      = 0


============================================================================
=
== ANALYSES AT THE INTRA-POPULATION LEVEL
============================================================================
=


============================================================================
==
== Sample :    Luci
============================================================================
==


================================
== Standard diversity indices : (Luci)
================================

Reference:
Nei, M., 1987.
No. of gene copies       : 20
No. of sequences         : 11
No. of loci              : 349
No. of usable loci       : 312 loci with less than  5.00 % missing data
No. of polymorphic sites : 9


Results are only shown for polymorphic loci
-------------------------------------------------
           Num.
           gene     Num.        Exp.
Locus#    copies   alleles       Het
-------------------------------------------------
    14        20         3   0.35263
    15        20         2   0.10000
    17        20         2   0.10000
    41        20         2   0.26842
    66        20         2   0.10000
   136        20         2   0.10000
   276        20         2   0.18947
   331        20         2   0.10000
   336        20         2   0.44211
-------------------------------------------------
  Mean    20.000     2.111   0.19474
  s.d.     0.000     0.333   0.13060
-------------------------------------------------


Haplotype-level computations
----------------------------

Sum of square freqs.     :  0.2000
Gene diversity           :  0.8421 +/-  0.0772
```

(Standard deviation is for the sampling process)

==========
== Molecular diversity indices : (Luci)
==========

Tajima, F., 1983.
Tajima, F. 1993.
Nei, M., 1987.
Zouros, E., 1979.
Ewens, W.J. 1972.

Sample size : 20.0000
No. of haplotypes : 11

Deletion weight : 1.0000
Transition weight : 1.0000
Transversion weight : 1.0000
Allowed level of missing data : 5.0000 %
Number of observed transitions : 9
Number of observed transversions : 1
Number of substitutions : 10
Number of observed indels : 0
Number of polymorphic sites : 9
Number of observed sites with transitions : 9
Number of observed sites with transversions : 1
Number of observed sites with substitutions : 9
Number of observed sites with indels : 0
Number of observed nucleotide sites : 349
Number of usable nucleotide sites : 312

Nucleotide composition (Relative values)
C : 15.76%
T : 45.20%
A : 30.07%
G : 8.98%
Total :100.00%

Distance method : Pairwise difference (no Gamma correction)

Inter-haplotypic distance matrix (s.d. above diagonal):

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H11	H12
H1		0.9984	1.7237	1.4097	0.9984	1.7237	1.7237	1.9871	1.4097	1.7237	1.7237
H2	1.0000		1.4097	0.9984	1.4097	1.4097	1.4097	1.7237	1.7237	1.9871	1.4097
H3	3.0000	2.0000		1.7237	1.9871	1.9871	1.9871	2.2181	2.2181	2.4258	1.9871
H4	2.0000	1.0000	3.0000		0.9984	0.9984	0.9984	1.4097	1.4097	1.7237	0.9984

```
      H5  1.0000  2.0000  4.0000  1.0000          1.4097  1.4097  1.7237
0.9984  1.4097  1.4097
      H6  3.0000  2.0000  4.0000  1.0000  2.0000          1.4097  0.9984
1.7237  1.9871  1.4097
      H7  3.0000  2.0000  4.0000  1.0000  2.0000  2.0000          1.7237
1.7237  1.9871  1.4097
      H8  4.0000  3.0000  5.0000  2.0000  3.0000  1.0000  3.0000
1.9871  2.2181  1.7237
      H9  2.0000  3.0000  5.0000  2.0000  1.0000  3.0000  3.0000  4.0000
0.9984  1.4097
     H11  3.0000  4.0000  6.0000  3.0000  2.0000  4.0000  4.0000  5.0000
1.0000          1.7237
     H12  3.0000  2.0000  4.0000  1.0000  2.0000  2.0000  2.0000  3.0000
2.0000  3.0000



List of Haplotypes:


      H1 :
CCCATATATTGGAGATACACTTACATCTTGAATTTGAGGAGGNNNNNNNNNNAATAATGCCACACTAACACGATTCT
TTTCTTTANNNNNNNATTCTTCCTTTTATTCTAATAATATTTATTATAATTCACATTCTTTTTCTTCATGAAACAGG
CAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAATTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAG
TAGGTTATTTAATTTTTTTTATATTATTTAATTATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAAT
TTTATTCCTGCTAATTCAATAGTAACCCCTATTCATATTC
      H2 :
CCCATATATTGGAGATACACTTACATCTTGAATTTGAGGAGGNNNNNNNNNNNNTAATGCCACACTAACACGATTCT
TTTCTTTANNNNNNNATTCTTCCTTTTATTCTAATAATATTTATTATAATTCACATTCTTTTTCTTCATGAAACAGG
CAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAATTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAG
TAGGTTATTTAATTTTTTTTATATTATTTAATTATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAAT
TTTATTCCTGCTAATTCAATAGTAACTCCTATTCATATTC
      H3 :
CCCATATATTGGAGATACACTTACATCTTGAATTTGAGGAGGNNNNNNAATTAATAATGCCACACTAACACGATTCT
TTTCTTTACANTTTTATTCTTCCTTTTATTCTAATAATATTTATTATAATTCACATTTTTTTTCTTCATGAAACAGG
CAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAATTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAG
TAGGTTATTTAATTTTTTTTATATTATTTAATTATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAAT
TTTATTCCTGCTAATTCAATAATAACTCCTATTCATATTC
      H4 :
CCCATATATTGGAGATACACTTACATCTTGAATTTGAGGGGGTNNNNNNNNNNNNAATGCCACACTAACACGATTCT
TTTCTTTACNNNNNNNNNNNNNCTTTTATTCTAATAATATTTATTATAATTCACATTCTTTTTCTTCATGAAACAGG
CAGAAGAAATCCTNNNNGTATCCCTTTAAACTTAGATAAAATTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAG
TAGGTTATTTAATTTTTTTTATATTATTTAATTATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAAT
TTTATTCCTGCTAATTCAATAGTAACTCCTATTCATATTC
      H5 :
CCCATATATTGGAGATACACTTACATCTTGAATTTGAGGGGNNNNNNNNNNNNAATAATGCCACACTAACACGATTCT
TTTCTTTANNNNNNTATTCTTCCTTTTATTCTAATAATATTTATTATAATTCACATTCTTTTTCTTCATGAAACAGG
CAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAATTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAG
TAGGTTATTTAATTTTTTTTATATTATTTAATTATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAAT
TTTATTCCTGCTAATTCAATAGTAACCCCTATTCATATTC
      H6 :
CCCATATATTGGAGATACACTTACATCTTGAATTTGAGGGGGNNNNNNNAATTAATAATGCCACACTAACACGATTCT
TTTCTTTACANTTTTATTCTTCCTTTTATTCTAATAATATTTATTATAATTCACATTCTTTTTCTTCATGAAACAGG
CAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAATTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAG
TAGGTTATTTAATTTTTTTTATATTATTTAATTATATTATTTTGTTAAATCCTTTTATATTTTTTGATCCTGATAAT
TTTATTCCTGCTAATTCAATAGTAACTCCTATTCATATTC
```

```
      H7 :
CCCATATATTGGAGACACACTTACATCTTGAATTTGAGGGGGNNNNNNAATTAATAATGCCACACTAACACGATTCT
TTTCTTTACANTTTTATTCTTCCTTTTATTCTAATAATATTTATTATAATTCACATTCTTTTTCTTCATGAAACAGG
CAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAATTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAG
TAGGTTATTTAATTTTTTTTATATTATTTAATTATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAAT
TTTATTCCTGCTAATTCAATAGTAACTCCTATTCATATTC
      H8 :
CCCATATATTGGAAATACACTTACATCTTGAATTTGAGGGGNNNNNNNNNNNNNTAATGCCACACTAACACGATTCT
TTTCTTTANNNNNNNNNNCTTCCTTTTATTCTAATAATATTTATTATAATTCACATTCTTTTTCTTCATGAAACAGG
CAGAAGAAATCCTTCAGGNATCCCTTTAAACTTAGATAAAATTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAG
TAGGTTNTTTAATTTTTTTTATATTATTTAATTATATTATTTTGTTAAATCCTTTTATATTTTTTGATCCTGATAAT
TTTATTCCTGCTAATTCAATAGTAACTCCTATTCATATTC
      H9 :
CCCATATATTGGGGATACACTTACATCTTGAATTTGAGGGGGNNNNNNNNNNNAATAATGCCACACTAACACGATTCT
TTTCTTTACANTTTTATTCTTCCTTTTATTCTAATAATATTTATTATAATTCACATTCTTTTTCTTCATGAAACAGG
CAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAATTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAG
TAGGTTATTTAATTTTTTTTATATTATTTAATTATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAAT
TTTATTCCTGCTAATTCAATAGTAACCCCTATTCATATTC
     H11 :
CCCATATATTGGGGATACACTTACATCTTGAATTTGAGGGGNNNNNNNNNNNNNTAATGCCACATTAACACGATTCT
TTTCTTTANNNNNNNNNTTCTTCCTTTTATTCTAATAATATTTATTATAATTCACATTCTTTTTCTTCATGAAACAGG
CAGAAGAAATNNNNNAGGTATCCCTTTAAACTTAGATAAAATTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAG
TAGGTTATTTAATTTTTTTTATATTATTTAATTATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAAT
TTTATTCCTGCTAATTCAATAGTAACCCCTATTCATATTC
     H12 :
CCCATATATTGGTGATACACTTACATCTTGAATTTGAGGGGGNNNNNNNNNTNAATAATGCCACACTAACACGATTCT
TTTCTTTACNNNNNNATTCTTCCTTTTATTCTAATAATATTTATTATAATTCACATTCTTTTTCTTCATGAAACAGG
CAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAATTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAG
TAGGTTATTTAATTTTTTTTATATTATTTAATTATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAAT
TTTATTCCTGCTAATTCAATAGTAACTCCTATTCATATTC
```

```
==========================================
MINIMUM SPANNING TREE between       11 OTUs
==========================================
Reference:
Rohlf, F.J., 1973.

   OTU 1     OTU 2    Connection length
   =====     =====    =================
      H1        H2              1.00000
      H2        H4              1.00000
      H1        H5              1.00000
      H4        H6              1.00000
      H4        H7              1.00000
      H6        H8              1.00000
      H5        H9              1.00000
      H9       H11              1.00000
      H4       H12              1.00000
      H2        H3              2.00000


------------------------------------------
NEXUS notation for MST
------------------------------------------
```

```
#NEXUS
begin trees;  [NEXUS Treefile section generated by Arlequin]
tree Luci_MST = [&U] ((H1:0, (H5:0, (H9:0, H11:1.00000):1.00000):1.00000):0,
((H2:0, H3:2.00000):0, (((H4:0, H12:1.00000):0, H7:1.00000):0, (H6:0,
H8:1.00000):1.00000):1.00000):1.00000);
end;



=====================================================================
Alternative connections between OTUs
to extend the minimum spanning tree into a MINIMUM SPANNING NETWORK
=====================================================================

     OTU    List of alternative links
     ===    =========================
      H4       H5 (1.00000)



Mean number of pairwise differences          :     1.752632 +/-     1.061515

Nucleotide diversity (average over loci)     :     0.005617 +/-     0.003800

(Standard deviations are for both the sampling and the stochastic processes)

       Theta(Hom) :     4.337593
  S.D. Theta(Hom) :     2.756248

         Theta(k) :     9.251987
95 % confidence interval limits for theta(k) : [     4.024408,    21.298908 ]

         Theta(S) :     2.536827
    S.D. Theta(S) :     1.165314

        Theta(Pi) :     1.752632
   S.D. Theta(Pi) :     1.185560


=============================================================================
==
== Sample :    Sept
=============================================================================
==


===============================
== Standard diversity indices : (Sept)
===============================

Reference:
Nei, M., 1987.
No. of gene copies       : 8
No. of sequences         : 2
No. of loci              : 349
```

```
No. of usable loci       : 320 loci with less than  5.00 % missing data
No. of polymorphic sites : 3


Results are only shown for polymorphic loci
-------------------------------------------------
           Num.
           gene     Num.        Exp.
Locus#    copies   alleles       Het
-------------------------------------------------
    14         8         2   0.25000
   240         8         2   0.25000
   336         8         2   0.25000
-------------------------------------------------
  Mean     8.000     2.000   0.25000
  s.d.     0.000     0.000   0.00000
-------------------------------------------------


Haplotype-level computations
----------------------------

Sum of square freqs.     :  0.7812
Gene diversity           :  0.2500 +/-  0.1802

(Standard deviation is for the sampling process)

================================
== Molecular diversity indices : (Sept)
================================

Tajima, F., 1983.
Tajima, F. 1993.
Nei, M., 1987.
Zouros, E., 1979.
Ewens, W.J. 1972.
Sample size                                 : 8.0000
No. of haplotypes                           : 2

Deletion weight                             : 1.0000
Transition weight                           : 1.0000
Transversion weight                         : 1.0000
Allowed level of missing data               : 5.0000 %
Number of observed transitions              : 3
Number of observed transversions            : 0
Number of substitutions                     : 3
Number of observed indels                   : 0
Number of polymorphic sites                 : 3
Number of observed sites with transitions   : 3
Number of observed sites with transversions : 0
Number of observed sites with substitutions : 3
Number of observed sites with indels        : 0
Number of observed nucleotide sites         : 349
Number of usable nucleotide sites           : 320

Nucleotide composition (Relative values)
   C : 15.73%
```

```
   T : 45.18%
   A : 29.99%
   G :  9.09%
   Total :100.00%


Distance method                              : Pairwise difference (no Gamma
correction)

Inter-haplotypic distance matrix (s.d. above diagonal):


              H4     H10

      H4          1.7239
     H10  3.0000



List of Haplotypes:


      H4 :
CCCATATATTGGAGATACACTTACATCTTGAATTTGAGGGGGTNNNNNNNNNNNNNAATGCCACACTAACACGATTCT
TTTCTTTACNNNNNNNNNNNNNCTTTTATTCTAATAATATTTATTATAATTCACATTCTTTTTCTTCATGAAACAGG
CAGAAGAAATCCTNNNNGTATCCCTTTAAACTTAGATAAAATTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAG
TAGGTTATTTAATTTTTTTTATATTATTTAATTATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAAT
TTTATTCCTGCTAATTCAATAGTAACTCCTATTCATATTC
     H10 :
CCCATATATTGGGGATACACTTACATCTTGAATTTGAGGGGGTTTNNNNNNTAATAATGCCACACTAACACGATTCT
TTTCTTTACANTTTTATTCTTCCTTTTATTCTAATAATATTTATTATAATTCACATTCTTTTTCTTCATGAAACAGG
CAGAAGAAATCCTTCAGGTATCCCTTTAAACTTAGATAAAATTCCATTTCATCCTTTTTTTAGACTAAAGGATTTAG
TAGGTTACTTAATTTTTTTTATATTATTTAATTATATTATTTTATTAAATCCTTTTATATTTTTTGATCCTGATAAT
TTTATTCCTGCTAATTCAATAGTAACCCCTATTCATATTC



==========================================
MINIMUM SPANNING TREE between        2 OTUs
==========================================
Reference:
Rohlf, F.J., 1973.

   OTU 1     OTU 2    Connection length
   =====     =====    =================
      H4       H10             3.00000


------------------------------------------
NEXUS notation for MST
------------------------------------------

#NEXUS
begin trees;  [NEXUS Treefile section generated by Arlequin]
tree Sept_MST = [&U] (H4:0, H10:3.00000);
end;
```

```
=================================================================
Alternative connections between OTUs
to extend the minimum spanning tree into a MINIMUM SPANNING NETWORK
=================================================================

     OTU    List of alternative links
     ===    =========================


Mean number of pairwise differences          :     0.750000 +/-     0.613775

Nucleotide diversity (average over loci)     :     0.002344 +/-     0.002185

(Standard deviations are for both the sampling and the stochastic processes)

       Theta(Hom) :     0.248412
  S.D. Theta(Hom) :     0.238007

         Theta(k) :     0.486761
95 % confidence interval limits for theta(k) : [     0.109246,     2.079968 ]

         Theta(S) :     1.157025
    S.D. Theta(S) :     0.781078

        Theta(Pi) :     0.750000
   S.D. Theta(Pi) :     0.699170


=============================================================================
===
== Summary of computations done within populations
=============================================================================
===


----------------
Basic properties
-----------------------------------------------------------------
          Statistics        Luci        Sept        Mean        s.d.
-----------------------------------------------------------------
  No. of gene copies          20           8      14.000       8.485
        No. of loci         349         349     349.000       0.000
  No. of usable loci        312         320     316.000       5.657
  No. of polym. loci          9           3       6.000       4.243
-----------------------------------------------------------------


-----------------------
Expected heterozygosity
-----------------------------------------------------------------
Locus#        Luci        Sept        Mean        s.d.   Tot. Het.
-----------------------------------------------------------------
```

1	0.00000	0.00000	0.00000	0.00000	0.00000
2	0.00000	0.00000	0.00000	0.00000	0.00000
3	0.00000	0.00000	0.00000	0.00000	0.00000
4	0.00000	0.00000	0.00000	0.00000	0.00000
5	0.00000	0.00000	0.00000	0.00000	0.00000
6	0.00000	0.00000	0.00000	0.00000	0.00000
7	0.00000	0.00000	0.00000	0.00000	0.00000
8	0.00000	0.00000	0.00000	0.00000	0.00000
9	0.00000	0.00000	0.00000	0.00000	0.00000
10	0.00000	0.00000	0.00000	0.00000	0.00000
11	0.00000	0.00000	0.00000	0.00000	0.00000
12	0.00000	0.00000	0.00000	0.00000	0.00000
13	0.00000	0.00000	0.00000	0.00000	0.00000
14	0.35263	0.25000	0.30132	0.07257	0.31481
15	0.10000	0.00000	0.05000	0.07071	0.07143
16	0.00000	0.00000	0.00000	0.00000	0.00000
17	0.10000	0.00000	0.05000	0.07071	0.07143
18	0.00000	0.00000	0.00000	0.00000	0.00000
19	0.00000	0.00000	0.00000	0.00000	0.00000
20	0.00000	0.00000	0.00000	0.00000	0.00000
21	0.00000	0.00000	0.00000	0.00000	0.00000
22	0.00000	0.00000	0.00000	0.00000	0.00000
23	0.00000	0.00000	0.00000	0.00000	0.00000
24	0.00000	0.00000	0.00000	0.00000	0.00000
25	0.00000	0.00000	0.00000	0.00000	0.00000
26	0.00000	0.00000	0.00000	0.00000	0.00000
27	0.00000	0.00000	0.00000	0.00000	0.00000
28	0.00000	0.00000	0.00000	0.00000	0.00000
29	0.00000	0.00000	0.00000	0.00000	0.00000
30	0.00000	0.00000	0.00000	0.00000	0.00000
31	0.00000	0.00000	0.00000	0.00000	0.00000
32	0.00000	0.00000	0.00000	0.00000	0.00000
33	0.00000	0.00000	0.00000	0.00000	0.00000
34	0.00000	0.00000	0.00000	0.00000	0.00000
35	0.00000	0.00000	0.00000	0.00000	0.00000
36	0.00000	0.00000	0.00000	0.00000	0.00000
37	0.00000	0.00000	0.00000	0.00000	0.00000
38	0.00000	0.00000	0.00000	0.00000	0.00000
39	0.00000	0.00000	0.00000	0.00000	0.00000
40	0.00000	0.00000	0.00000	0.00000	0.00000
41	0.26842	0.00000	0.13421	0.18980	0.19841
42	0.00000	0.00000	0.00000	0.00000	0.00000
43	0.00000	0.00000	0.00000	0.00000	0.00000
44	0.00000	0.00000	0.00000	0.00000	0.00000
45	0.00000	0.00000	0.00000	0.00000	0.00000
46	0.00000	0.00000	0.00000	0.00000	0.00000
47	0.00000	0.00000	0.00000	0.00000	0.00000
48	0.00000	0.00000	0.00000	0.00000	0.00000
49	0.00000	0.00000	0.00000	0.00000	0.00000
50	0.00000	0.00000	0.00000	0.00000	0.00000
51	0.00000	0.00000	0.00000	0.00000	0.00000
52	0.00000	0.00000	0.00000	0.00000	0.00000
53	0.00000	0.00000	0.00000	0.00000	0.00000
54	0.00000	0.00000	0.00000	0.00000	0.00000
55	0.00000	0.00000	0.00000	0.00000	0.00000
56	0.00000	0.00000	0.00000	0.00000	0.00000
57	0.00000	0.00000	0.00000	0.00000	0.00000

58	0.00000	0.00000	0.00000	0.00000	0.00000
59	0.00000	0.00000	0.00000	0.00000	0.00000
60	0.00000	0.00000	0.00000	0.00000	0.00000
61	0.00000	0.00000	0.00000	0.00000	0.00000
62	0.00000	0.00000	0.00000	0.00000	0.00000
63	0.00000	0.00000	0.00000	0.00000	0.00000
64	0.00000	0.00000	0.00000	0.00000	0.00000
65	0.00000	0.00000	0.00000	0.00000	0.00000
66	0.10000	0.00000	0.05000	0.07071	0.07143
67	0.00000	0.00000	0.00000	0.00000	0.00000
68	0.00000	0.00000	0.00000	0.00000	0.00000
69	0.00000	0.00000	0.00000	0.00000	0.00000
70	0.00000	0.00000	0.00000	0.00000	0.00000
71	0.00000	0.00000	0.00000	0.00000	0.00000
72	0.00000	0.00000	0.00000	0.00000	0.00000
73	0.00000	0.00000	0.00000	0.00000	0.00000
74	0.00000	0.00000	0.00000	0.00000	0.00000
75	0.00000	0.00000	0.00000	0.00000	0.00000
76	0.00000	0.00000	0.00000	0.00000	0.00000
77	0.00000	0.00000	0.00000	0.00000	0.00000
78	0.00000	0.00000	0.00000	0.00000	0.00000
79	0.00000	0.00000	0.00000	0.00000	0.00000
80	0.00000	0.00000	0.00000	0.00000	0.00000
81	0.00000	0.00000	0.00000	0.00000	0.00000
82	0.00000	0.00000	0.00000	0.00000	0.00000
83	0.00000	0.00000	0.00000	0.00000	0.00000
84	0.00000	0.00000	0.00000	0.00000	0.00000
85	0.00000	0.00000	0.00000	0.00000	0.00000
86	0.00000	0.00000	0.00000	0.00000	0.00000
87	0.00000	0.00000	0.00000	0.00000	0.00000
88	0.00000	0.00000	0.00000	0.00000	0.00000
89	0.00000	0.00000	0.00000	0.00000	0.00000
90	0.00000	0.00000	0.00000	0.00000	0.00000
91	0.00000	0.00000	0.00000	0.00000	0.00000
92	0.00000	0.00000	0.00000	0.00000	0.00000
93	0.00000	0.00000	0.00000	0.00000	0.00000
94	0.00000	0.00000	0.00000	0.00000	0.00000
95	0.00000	0.00000	0.00000	0.00000	0.00000
96	0.00000	0.00000	0.00000	0.00000	0.00000
97	0.00000	0.00000	0.00000	0.00000	0.00000
98	0.00000	0.00000	0.00000	0.00000	0.00000
99	0.00000	0.00000	0.00000	0.00000	0.00000
100	0.00000	0.00000	0.00000	0.00000	0.00000
101	0.00000	0.00000	0.00000	0.00000	0.00000
102	0.00000	0.00000	0.00000	0.00000	0.00000
103	0.00000	0.00000	0.00000	0.00000	0.00000
104	0.00000	0.00000	0.00000	0.00000	0.00000
105	0.00000	0.00000	0.00000	0.00000	0.00000
106	0.00000	0.00000	0.00000	0.00000	0.00000
107	0.00000	0.00000	0.00000	0.00000	0.00000
108	0.00000	0.00000	0.00000	0.00000	0.00000
109	0.00000	0.00000	0.00000	0.00000	0.00000
110	0.00000	0.00000	0.00000	0.00000	0.00000
111	0.00000	0.00000	0.00000	0.00000	0.00000
112	0.00000	0.00000	0.00000	0.00000	0.00000
113	0.00000	0.00000	0.00000	0.00000	0.00000
114	0.00000	0.00000	0.00000	0.00000	0.00000

115	0.00000	0.00000	0.00000	0.00000	0.00000
116	0.00000	0.00000	0.00000	0.00000	0.00000
117	0.00000	0.00000	0.00000	0.00000	0.00000
118	0.00000	0.00000	0.00000	0.00000	0.00000
119	0.00000	0.00000	0.00000	0.00000	0.00000
120	0.00000	0.00000	0.00000	0.00000	0.00000
121	0.00000	0.00000	0.00000	0.00000	0.00000
122	0.00000	0.00000	0.00000	0.00000	0.00000
123	0.00000	0.00000	0.00000	0.00000	0.00000
124	0.00000	0.00000	0.00000	0.00000	0.00000
125	0.00000	0.00000	0.00000	0.00000	0.00000
126	0.00000	0.00000	0.00000	0.00000	0.00000
127	0.00000	0.00000	0.00000	0.00000	0.00000
128	0.00000	0.00000	0.00000	0.00000	0.00000
129	0.00000	0.00000	0.00000	0.00000	0.00000
130	0.00000	0.00000	0.00000	0.00000	0.00000
131	0.00000	0.00000	0.00000	0.00000	0.00000
132	0.00000	0.00000	0.00000	0.00000	0.00000
133	0.00000	0.00000	0.00000	0.00000	0.00000
134	0.00000	0.00000	0.00000	0.00000	0.00000
135	0.00000	0.00000	0.00000	0.00000	0.00000
136	0.10000	0.00000	0.05000	0.07071	0.07143
137	0.00000	0.00000	0.00000	0.00000	0.00000
138	0.00000	0.00000	0.00000	0.00000	0.00000
139	0.00000	0.00000	0.00000	0.00000	0.00000
140	0.00000	0.00000	0.00000	0.00000	0.00000
141	0.00000	0.00000	0.00000	0.00000	0.00000
142	0.00000	0.00000	0.00000	0.00000	0.00000
143	0.00000	0.00000	0.00000	0.00000	0.00000
144	0.00000	0.00000	0.00000	0.00000	0.00000
145	0.00000	0.00000	0.00000	0.00000	0.00000
146	0.00000	0.00000	0.00000	0.00000	0.00000
147	0.00000	0.00000	0.00000	0.00000	0.00000
148	0.00000	0.00000	0.00000	0.00000	0.00000
149	0.00000	0.00000	0.00000	0.00000	0.00000
150	0.00000	0.00000	0.00000	0.00000	0.00000
151	0.00000	0.00000	0.00000	0.00000	0.00000
152	0.00000	0.00000	0.00000	0.00000	0.00000
153	0.00000	0.00000	0.00000	0.00000	0.00000
154	0.00000	0.00000	0.00000	0.00000	0.00000
155	0.00000	0.00000	0.00000	0.00000	0.00000
156	0.00000	0.00000	0.00000	0.00000	0.00000
157	0.00000	0.00000	0.00000	0.00000	0.00000
158	0.00000	0.00000	0.00000	0.00000	0.00000
159	0.00000	0.00000	0.00000	0.00000	0.00000
160	0.00000	0.00000	0.00000	0.00000	0.00000
161	0.00000	0.00000	0.00000	0.00000	0.00000
162	0.00000	0.00000	0.00000	0.00000	0.00000
163	0.00000	0.00000	0.00000	0.00000	0.00000
164	0.00000	0.00000	0.00000	0.00000	0.00000
165	0.00000	0.00000	0.00000	0.00000	0.00000
166	0.00000	0.00000	0.00000	0.00000	0.00000
167	0.00000	0.00000	0.00000	0.00000	0.00000
168	0.00000	0.00000	0.00000	0.00000	0.00000
169	0.00000	0.00000	0.00000	0.00000	0.00000
170	0.00000	0.00000	0.00000	0.00000	0.00000
171	0.00000	0.00000	0.00000	0.00000	0.00000

172	0.00000	0.00000	0.00000	0.00000	0.00000
173	0.00000	0.00000	0.00000	0.00000	0.00000
174	0.00000	0.00000	0.00000	0.00000	0.00000
175	0.00000	0.00000	0.00000	0.00000	0.00000
176	0.00000	0.00000	0.00000	0.00000	0.00000
177	0.00000	0.00000	0.00000	0.00000	0.00000
178	0.00000	0.00000	0.00000	0.00000	0.00000
179	0.00000	0.00000	0.00000	0.00000	0.00000
180	0.00000	0.00000	0.00000	0.00000	0.00000
181	0.00000	0.00000	0.00000	0.00000	0.00000
182	0.00000	0.00000	0.00000	0.00000	0.00000
183	0.00000	0.00000	0.00000	0.00000	0.00000
184	0.00000	0.00000	0.00000	0.00000	0.00000
185	0.00000	0.00000	0.00000	0.00000	0.00000
186	0.00000	0.00000	0.00000	0.00000	0.00000
187	0.00000	0.00000	0.00000	0.00000	0.00000
188	0.00000	0.00000	0.00000	0.00000	0.00000
189	0.00000	0.00000	0.00000	0.00000	0.00000
190	0.00000	0.00000	0.00000	0.00000	0.00000
191	0.00000	0.00000	0.00000	0.00000	0.00000
192	0.00000	0.00000	0.00000	0.00000	0.00000
193	0.00000	0.00000	0.00000	0.00000	0.00000
194	0.00000	0.00000	0.00000	0.00000	0.00000
195	0.00000	0.00000	0.00000	0.00000	0.00000
196	0.00000	0.00000	0.00000	0.00000	0.00000
197	0.00000	0.00000	0.00000	0.00000	0.00000
198	0.00000	0.00000	0.00000	0.00000	0.00000
199	0.00000	0.00000	0.00000	0.00000	0.00000
200	0.00000	0.00000	0.00000	0.00000	0.00000
201	0.00000	0.00000	0.00000	0.00000	0.00000
202	0.00000	0.00000	0.00000	0.00000	0.00000
203	0.00000	0.00000	0.00000	0.00000	0.00000
204	0.00000	0.00000	0.00000	0.00000	0.00000
205	0.00000	0.00000	0.00000	0.00000	0.00000
206	0.00000	0.00000	0.00000	0.00000	0.00000
207	0.00000	0.00000	0.00000	0.00000	0.00000
208	0.00000	0.00000	0.00000	0.00000	0.00000
209	0.00000	0.00000	0.00000	0.00000	0.00000
210	0.00000	0.00000	0.00000	0.00000	0.00000
211	0.00000	0.00000	0.00000	0.00000	0.00000
212	0.00000	0.00000	0.00000	0.00000	0.00000
213	0.00000	0.00000	0.00000	0.00000	0.00000
214	0.00000	0.00000	0.00000	0.00000	0.00000
215	0.00000	0.00000	0.00000	0.00000	0.00000
216	0.00000	0.00000	0.00000	0.00000	0.00000
217	0.00000	0.00000	0.00000	0.00000	0.00000
218	0.00000	0.00000	0.00000	0.00000	0.00000
219	0.00000	0.00000	0.00000	0.00000	0.00000
220	0.00000	0.00000	0.00000	0.00000	0.00000
221	0.00000	0.00000	0.00000	0.00000	0.00000
222	0.00000	0.00000	0.00000	0.00000	0.00000
223	0.00000	0.00000	0.00000	0.00000	0.00000
224	0.00000	0.00000	0.00000	0.00000	0.00000
225	0.00000	0.00000	0.00000	0.00000	0.00000
226	0.00000	0.00000	0.00000	0.00000	0.00000
227	0.00000	0.00000	0.00000	0.00000	0.00000
228	0.00000	0.00000	0.00000	0.00000	0.00000

229	0.00000	0.00000	0.00000	0.00000	0.00000
230	0.00000	0.00000	0.00000	0.00000	0.00000
231	0.00000	0.00000	0.00000	0.00000	0.00000
232	0.00000	0.00000	0.00000	0.00000	0.00000
233	0.00000	0.00000	0.00000	0.00000	0.00000
234	0.00000	0.00000	0.00000	0.00000	0.00000
235	0.00000	0.00000	0.00000	0.00000	0.00000
236	0.00000	0.00000	0.00000	0.00000	0.00000
237	0.00000	0.00000	0.00000	0.00000	0.00000
238	0.00000	0.00000	0.00000	0.00000	0.00000
239	0.00000	0.00000	0.00000	0.00000	0.00000
240	0.00000	0.25000	0.12500	0.17678	0.07143
241	0.00000	0.00000	0.00000	0.00000	0.00000
242	0.00000	0.00000	0.00000	0.00000	0.00000
243	0.00000	0.00000	0.00000	0.00000	0.00000
244	0.00000	0.00000	0.00000	0.00000	0.00000
245	0.00000	0.00000	0.00000	0.00000	0.00000
246	0.00000	0.00000	0.00000	0.00000	0.00000
247	0.00000	0.00000	0.00000	0.00000	0.00000
248	0.00000	0.00000	0.00000	0.00000	0.00000
249	0.00000	0.00000	0.00000	0.00000	0.00000
250	0.00000	0.00000	0.00000	0.00000	0.00000
251	0.00000	0.00000	0.00000	0.00000	0.00000
252	0.00000	0.00000	0.00000	0.00000	0.00000
253	0.00000	0.00000	0.00000	0.00000	0.00000
254	0.00000	0.00000	0.00000	0.00000	0.00000
255	0.00000	0.00000	0.00000	0.00000	0.00000
256	0.00000	0.00000	0.00000	0.00000	0.00000
257	0.00000	0.00000	0.00000	0.00000	0.00000
258	0.00000	0.00000	0.00000	0.00000	0.00000
259	0.00000	0.00000	0.00000	0.00000	0.00000
260	0.00000	0.00000	0.00000	0.00000	0.00000
261	0.00000	0.00000	0.00000	0.00000	0.00000
262	0.00000	0.00000	0.00000	0.00000	0.00000
263	0.00000	0.00000	0.00000	0.00000	0.00000
264	0.00000	0.00000	0.00000	0.00000	0.00000
265	0.00000	0.00000	0.00000	0.00000	0.00000
266	0.00000	0.00000	0.00000	0.00000	0.00000
267	0.00000	0.00000	0.00000	0.00000	0.00000
268	0.00000	0.00000	0.00000	0.00000	0.00000
269	0.00000	0.00000	0.00000	0.00000	0.00000
270	0.00000	0.00000	0.00000	0.00000	0.00000
271	0.00000	0.00000	0.00000	0.00000	0.00000
272	0.00000	0.00000	0.00000	0.00000	0.00000
273	0.00000	0.00000	0.00000	0.00000	0.00000
274	0.00000	0.00000	0.00000	0.00000	0.00000
275	0.00000	0.00000	0.00000	0.00000	0.00000
276	0.18947	0.00000	0.09474	0.13398	0.13757
277	0.00000	0.00000	0.00000	0.00000	0.00000
278	0.00000	0.00000	0.00000	0.00000	0.00000
279	0.00000	0.00000	0.00000	0.00000	0.00000
280	0.00000	0.00000	0.00000	0.00000	0.00000
281	0.00000	0.00000	0.00000	0.00000	0.00000
282	0.00000	0.00000	0.00000	0.00000	0.00000
283	0.00000	0.00000	0.00000	0.00000	0.00000
284	0.00000	0.00000	0.00000	0.00000	0.00000
285	0.00000	0.00000	0.00000	0.00000	0.00000

286	0.00000	0.00000	0.00000	0.00000	0.00000
287	0.00000	0.00000	0.00000	0.00000	0.00000
288	0.00000	0.00000	0.00000	0.00000	0.00000
289	0.00000	0.00000	0.00000	0.00000	0.00000
290	0.00000	0.00000	0.00000	0.00000	0.00000
291	0.00000	0.00000	0.00000	0.00000	0.00000
292	0.00000	0.00000	0.00000	0.00000	0.00000
293	0.00000	0.00000	0.00000	0.00000	0.00000
294	0.00000	0.00000	0.00000	0.00000	0.00000
295	0.00000	0.00000	0.00000	0.00000	0.00000
296	0.00000	0.00000	0.00000	0.00000	0.00000
297	0.00000	0.00000	0.00000	0.00000	0.00000
298	0.00000	0.00000	0.00000	0.00000	0.00000
299	0.00000	0.00000	0.00000	0.00000	0.00000
300	0.00000	0.00000	0.00000	0.00000	0.00000
301	0.00000	0.00000	0.00000	0.00000	0.00000
302	0.00000	0.00000	0.00000	0.00000	0.00000
303	0.00000	0.00000	0.00000	0.00000	0.00000
304	0.00000	0.00000	0.00000	0.00000	0.00000
305	0.00000	0.00000	0.00000	0.00000	0.00000
306	0.00000	0.00000	0.00000	0.00000	0.00000
307	0.00000	0.00000	0.00000	0.00000	0.00000
308	0.00000	0.00000	0.00000	0.00000	0.00000
309	0.00000	0.00000	0.00000	0.00000	0.00000
310	0.00000	0.00000	0.00000	0.00000	0.00000
311	0.00000	0.00000	0.00000	0.00000	0.00000
312	0.00000	0.00000	0.00000	0.00000	0.00000
313	0.00000	0.00000	0.00000	0.00000	0.00000
314	0.00000	0.00000	0.00000	0.00000	0.00000
315	0.00000	0.00000	0.00000	0.00000	0.00000
316	0.00000	0.00000	0.00000	0.00000	0.00000
317	0.00000	0.00000	0.00000	0.00000	0.00000
318	0.00000	0.00000	0.00000	0.00000	0.00000
319	0.00000	0.00000	0.00000	0.00000	0.00000
320	0.00000	0.00000	0.00000	0.00000	0.00000
321	0.00000	0.00000	0.00000	0.00000	0.00000
322	0.00000	0.00000	0.00000	0.00000	0.00000
323	0.00000	0.00000	0.00000	0.00000	0.00000
324	0.00000	0.00000	0.00000	0.00000	0.00000
325	0.00000	0.00000	0.00000	0.00000	0.00000
326	0.00000	0.00000	0.00000	0.00000	0.00000
327	0.00000	0.00000	0.00000	0.00000	0.00000
328	0.00000	0.00000	0.00000	0.00000	0.00000
329	0.00000	0.00000	0.00000	0.00000	0.00000
330	0.00000	0.00000	0.00000	0.00000	0.00000
331	0.10000	0.00000	0.05000	0.07071	0.07143
332	0.00000	0.00000	0.00000	0.00000	0.00000
333	0.00000	0.00000	0.00000	0.00000	0.00000
334	0.00000	0.00000	0.00000	0.00000	0.00000
335	0.00000	0.00000	0.00000	0.00000	0.00000
336	0.44211	0.25000	0.34605	0.13584	0.38889
337	0.00000	0.00000	0.00000	0.00000	0.00000
338	0.00000	0.00000	0.00000	0.00000	0.00000
339	0.00000	0.00000	0.00000	0.00000	0.00000
340	0.00000	0.00000	0.00000	0.00000	0.00000
341	0.00000	0.00000	0.00000	0.00000	0.00000
342	0.00000	0.00000	0.00000	0.00000	0.00000

343	0.00000	0.00000	0.00000	0.00000	0.00000
344	0.00000	0.00000	0.00000	0.00000	0.00000
345	0.00000	0.00000	0.00000	0.00000	0.00000
346	0.00000	0.00000	0.00000	0.00000	0.00000
347	0.00000	0.00000	0.00000	0.00000	0.00000
348	0.00000	0.00000	0.00000	0.00000	0.00000
349	0.00000	0.00000	0.00000	0.00000	0.00000
Mean	0.00502	0.00215	0.00359	0.00203	0.14683
s.d.	0.03671	0.02311	0.02991	0.00961	0.11722

Number of alleles

Locus#	Luci	Sept	Mean	s.d.	Tot. number
1	1	1	1.000	0.000	1
2	1	1	1.000	0.000	1
3	1	1	1.000	0.000	1
4	1	1	1.000	0.000	1
5	1	1	1.000	0.000	1
6	1	1	1.000	0.000	1
7	1	1	1.000	0.000	1
8	1	1	1.000	0.000	1
9	1	1	1.000	0.000	1
10	1	1	1.000	0.000	1
11	1	1	1.000	0.000	1
12	1	1	1.000	0.000	1
13	1	1	1.000	0.000	1
14	3	2	2.500	0.707	3
15	2	1	1.500	0.707	2
16	1	1	1.000	0.000	1
17	2	1	1.500	0.707	2
18	1	1	1.000	0.000	1
19	1	1	1.000	0.000	1
20	1	1	1.000	0.000	1
21	1	1	1.000	0.000	1
22	1	1	1.000	0.000	1
23	1	1	1.000	0.000	1
24	1	1	1.000	0.000	1
25	1	1	1.000	0.000	1
26	1	1	1.000	0.000	1
27	1	1	1.000	0.000	1
28	1	1	1.000	0.000	1
29	1	1	1.000	0.000	1
30	1	1	1.000	0.000	1
31	1	1	1.000	0.000	1
32	1	1	1.000	0.000	1
33	1	1	1.000	0.000	1
34	1	1	1.000	0.000	1
35	1	1	1.000	0.000	1
36	1	1	1.000	0.000	1
37	1	1	1.000	0.000	1
38	1	1	1.000	0.000	1

39	1	1	1.000	0.000	1
40	1	1	1.000	0.000	1
41	2	1	1.500	0.707	2
42	1	1	1.000	0.000	1
43	1	1	1.000	0.000	1
44	1	1	1.000	0.000	1
45	0	1	0.500	0.707	1
46	0	1	0.500	0.707	1
47	0	0	0.000	0.000	0
48	0	0	0.000	0.000	0
49	0	0	0.000	0.000	0
50	1	0	0.500	0.707	1
51	1	0	0.500	0.707	1
52	1	0	0.500	0.707	1
53	1	1	1.000	0.000	1
54	1	1	1.000	0.000	1
55	1	1	1.000	0.000	1
56	1	1	1.000	0.000	1
57	1	1	1.000	0.000	1
58	1	1	1.000	0.000	1
59	1	1	1.000	0.000	1
60	1	1	1.000	0.000	1
61	1	1	1.000	0.000	1
62	1	1	1.000	0.000	1
63	1	1	1.000	0.000	1
64	1	1	1.000	0.000	1
65	1	1	1.000	0.000	1
66	2	1	1.500	0.707	2
67	1	1	1.000	0.000	1
68	1	1	1.000	0.000	1
69	1	1	1.000	0.000	1
70	1	1	1.000	0.000	1
71	1	1	1.000	0.000	1
72	1	1	1.000	0.000	1
73	1	1	1.000	0.000	1
74	1	1	1.000	0.000	1
75	1	1	1.000	0.000	1
76	1	1	1.000	0.000	1
77	1	1	1.000	0.000	1
78	1	1	1.000	0.000	1
79	1	1	1.000	0.000	1
80	1	1	1.000	0.000	1
81	1	1	1.000	0.000	1
82	1	1	1.000	0.000	1
83	1	1	1.000	0.000	1
84	1	1	1.000	0.000	1
85	1	1	1.000	0.000	1
86	1	1	1.000	0.000	1
87	1	1	1.000	0.000	1
88	1	1	1.000	0.000	1
89	0	0	0.000	0.000	0
90	1	1	1.000	0.000	1
91	1	1	1.000	0.000	1
92	1	1	1.000	0.000	1
93	1	1	1.000	0.000	1
94	1	1	1.000	0.000	1
95	1	1	1.000	0.000	1

96	1	1	1.000	0.000	1
97	1	1	1.000	0.000	1
98	1	1	1.000	0.000	1
99	1	1	1.000	0.000	1
100	1	1	1.000	0.000	1
101	1	1	1.000	0.000	1
102	1	1	1.000	0.000	1
103	1	1	1.000	0.000	1
104	1	1	1.000	0.000	1
105	1	1	1.000	0.000	1
106	1	1	1.000	0.000	1
107	1	1	1.000	0.000	1
108	1	1	1.000	0.000	1
109	1	1	1.000	0.000	1
110	1	1	1.000	0.000	1
111	1	1	1.000	0.000	1
112	1	1	1.000	0.000	1
113	1	1	1.000	0.000	1
114	1	1	1.000	0.000	1
115	1	1	1.000	0.000	1
116	1	1	1.000	0.000	1
117	1	1	1.000	0.000	1
118	1	1	1.000	0.000	1
119	1	1	1.000	0.000	1
120	1	1	1.000	0.000	1
121	1	1	1.000	0.000	1
122	1	1	1.000	0.000	1
123	1	1	1.000	0.000	1
124	1	1	1.000	0.000	1
125	1	1	1.000	0.000	1
126	1	1	1.000	0.000	1
127	1	1	1.000	0.000	1
128	1	1	1.000	0.000	1
129	1	1	1.000	0.000	1
130	1	1	1.000	0.000	1
131	1	1	1.000	0.000	1
132	1	1	1.000	0.000	1
133	1	1	1.000	0.000	1
134	1	1	1.000	0.000	1
135	1	1	1.000	0.000	1
136	2	1	1.500	0.707	2
137	1	1	1.000	0.000	1
138	1	1	1.000	0.000	1
139	1	1	1.000	0.000	1
140	1	1	1.000	0.000	1
141	1	1	1.000	0.000	1
142	1	1	1.000	0.000	1
143	1	1	1.000	0.000	1
144	1	1	1.000	0.000	1
145	1	1	1.000	0.000	1
146	1	1	1.000	0.000	1
147	1	1	1.000	0.000	1
148	1	1	1.000	0.000	1
149	1	1	1.000	0.000	1
150	1	1	1.000	0.000	1
151	1	1	1.000	0.000	1
152	1	1	1.000	0.000	1

153	1	1	1.000	0.000	1
154	1	1	1.000	0.000	1
155	1	1	1.000	0.000	1
156	1	1	1.000	0.000	1
157	1	1	1.000	0.000	1
158	1	1	1.000	0.000	1
159	1	1	1.000	0.000	1
160	1	1	1.000	0.000	1
161	1	1	1.000	0.000	1
162	1	1	1.000	0.000	1
163	1	1	1.000	0.000	1
164	1	1	1.000	0.000	1
165	1	1	1.000	0.000	1
166	1	1	1.000	0.000	1
167	1	1	1.000	0.000	1
168	1	1	1.000	0.000	1
169	1	1	1.000	0.000	1
170	1	1	1.000	0.000	1
171	1	1	1.000	0.000	1
172	1	1	1.000	0.000	1
173	1	1	1.000	0.000	1
174	1	1	1.000	0.000	1
175	1	1	1.000	0.000	1
176	1	1	1.000	0.000	1
177	1	1	1.000	0.000	1
178	1	1	1.000	0.000	1
179	1	1	1.000	0.000	1
180	1	1	1.000	0.000	1
181	1	1	1.000	0.000	1
182	1	1	1.000	0.000	1
183	1	1	1.000	0.000	1
184	1	1	1.000	0.000	1
185	1	1	1.000	0.000	1
186	1	1	1.000	0.000	1
187	1	1	1.000	0.000	1
188	1	1	1.000	0.000	1
189	1	1	1.000	0.000	1
190	1	1	1.000	0.000	1
191	1	1	1.000	0.000	1
192	1	1	1.000	0.000	1
193	1	1	1.000	0.000	1
194	1	1	1.000	0.000	1
195	1	1	1.000	0.000	1
196	1	1	1.000	0.000	1
197	1	1	1.000	0.000	1
198	1	1	1.000	0.000	1
199	1	1	1.000	0.000	1
200	1	1	1.000	0.000	1
201	1	1	1.000	0.000	1
202	1	1	1.000	0.000	1
203	1	1	1.000	0.000	1
204	1	1	1.000	0.000	1
205	1	1	1.000	0.000	1
206	1	1	1.000	0.000	1
207	1	1	1.000	0.000	1
208	1	1	1.000	0.000	1
209	1	1	1.000	0.000	1

210	1	1	1.000	0.000	1
211	1	1	1.000	0.000	1
212	1	1	1.000	0.000	1
213	1	1	1.000	0.000	1
214	1	1	1.000	0.000	1
215	1	1	1.000	0.000	1
216	1	1	1.000	0.000	1
217	1	1	1.000	0.000	1
218	1	1	1.000	0.000	1
219	1	1	1.000	0.000	1
220	1	1	1.000	0.000	1
221	1	1	1.000	0.000	1
222	1	1	1.000	0.000	1
223	1	1	1.000	0.000	1
224	1	1	1.000	0.000	1
225	1	1	1.000	0.000	1
226	1	1	1.000	0.000	1
227	1	1	1.000	0.000	1
228	1	1	1.000	0.000	1
229	1	1	1.000	0.000	1
230	1	1	1.000	0.000	1
231	1	1	1.000	0.000	1
232	1	1	1.000	0.000	1
233	1	1	1.000	0.000	1
234	1	1	1.000	0.000	1
235	1	1	1.000	0.000	1
236	1	1	1.000	0.000	1
237	1	1	1.000	0.000	1
238	1	1	1.000	0.000	1
239	1	1	1.000	0.000	1
240	1	2	1.500	0.707	2
241	1	1	1.000	0.000	1
242	1	1	1.000	0.000	1
243	1	1	1.000	0.000	1
244	1	1	1.000	0.000	1
245	1	1	1.000	0.000	1
246	1	1	1.000	0.000	1
247	1	1	1.000	0.000	1
248	1	1	1.000	0.000	1
249	1	1	1.000	0.000	1
250	1	1	1.000	0.000	1
251	1	1	1.000	0.000	1
252	1	1	1.000	0.000	1
253	1	1	1.000	0.000	1
254	1	1	1.000	0.000	1
255	1	1	1.000	0.000	1
256	1	1	1.000	0.000	1
257	1	1	1.000	0.000	1
258	1	1	1.000	0.000	1
259	1	1	1.000	0.000	1
260	1	1	1.000	0.000	1
261	1	1	1.000	0.000	1
262	1	1	1.000	0.000	1
263	1	1	1.000	0.000	1
264	1	1	1.000	0.000	1
265	1	1	1.000	0.000	1
266	1	1	1.000	0.000	1

267	1	1	1.000	0.000	1
268	1	1	1.000	0.000	1
269	1	1	1.000	0.000	1
270	1	1	1.000	0.000	1
271	1	1	1.000	0.000	1
272	1	1	1.000	0.000	1
273	1	1	1.000	0.000	1
274	1	1	1.000	0.000	1
275	1	1	1.000	0.000	1
276	2	1	1.500	0.707	2
277	1	1	1.000	0.000	1
278	1	1	1.000	0.000	1
279	1	1	1.000	0.000	1
280	1	1	1.000	0.000	1
281	1	1	1.000	0.000	1
282	1	1	1.000	0.000	1
283	1	1	1.000	0.000	1
284	1	1	1.000	0.000	1
285	1	1	1.000	0.000	1
286	1	1	1.000	0.000	1
287	1	1	1.000	0.000	1
288	1	1	1.000	0.000	1
289	1	1	1.000	0.000	1
290	1	1	1.000	0.000	1
291	1	1	1.000	0.000	1
292	1	1	1.000	0.000	1
293	1	1	1.000	0.000	1
294	1	1	1.000	0.000	1
295	1	1	1.000	0.000	1
296	1	1	1.000	0.000	1
297	1	1	1.000	0.000	1
298	1	1	1.000	0.000	1
299	1	1	1.000	0.000	1
300	1	1	1.000	0.000	1
301	1	1	1.000	0.000	1
302	1	1	1.000	0.000	1
303	1	1	1.000	0.000	1
304	1	1	1.000	0.000	1
305	1	1	1.000	0.000	1
306	1	1	1.000	0.000	1
307	1	1	1.000	0.000	1
308	1	1	1.000	0.000	1
309	1	1	1.000	0.000	1
310	1	1	1.000	0.000	1
311	1	1	1.000	0.000	1
312	1	1	1.000	0.000	1
313	1	1	1.000	0.000	1
314	1	1	1.000	0.000	1
315	1	1	1.000	0.000	1
316	1	1	1.000	0.000	1
317	1	1	1.000	0.000	1
318	1	1	1.000	0.000	1
319	1	1	1.000	0.000	1
320	1	1	1.000	0.000	1
321	1	1	1.000	0.000	1
322	1	1	1.000	0.000	1
323	1	1	1.000	0.000	1

324	1	1	1.000	0.000	1
325	1	1	1.000	0.000	1
326	1	1	1.000	0.000	1
327	1	1	1.000	0.000	1
328	1	1	1.000	0.000	1
329	1	1	1.000	0.000	1
330	1	1	1.000	0.000	1
331	2	1	1.500	0.707	2
332	1	1	1.000	0.000	1
333	1	1	1.000	0.000	1
334	1	1	1.000	0.000	1
335	1	1	1.000	0.000	1
336	2	2	2.000	0.000	2
337	1	1	1.000	0.000	1
338	1	1	1.000	0.000	1
339	1	1	1.000	0.000	1
340	1	1	1.000	0.000	1
341	1	1	1.000	0.000	1
342	1	1	1.000	0.000	1
343	1	1	1.000	0.000	1
344	1	1	1.000	0.000	1
345	1	1	1.000	0.000	1
346	1	1	1.000	0.000	1
347	1	1	1.000	0.000	1
348	1	1	1.000	0.000	1
349	1	1	1.000	0.000	1
Mean	1.011	0.989	1.000	0.016	1.020
s.d.	0.227	0.169	0.198	0.041	0.316

Molecular diversity indexes

Statistics	Luci	Sept	Mean	s.d.
No. of transitions	9	3	6.000	4.243
No. of transversions	1	0	0.500	0.707
No. of substitutions	10	3	6.500	4.950
No. of indels	0	0	0.000	0.000
No. of ts. sites	9	3	6.000	4.243
No. of tv. sites	1	0	0.500	0.707
No. of subst. sites	9	3	6.000	4.243
Total: 10				
No. private subst. sites	7	1	4.000	4.243
No. of indel sites	0	0	0.000	0.000
Pi	1.753	0.750	1.25132	0.70897
Theta_k	9.25199	0.48676	4.86937	6.19795
Theta_k_lower	4.02441	0.10925	2.06683	2.76844
Theta_k_upper	21.29891	2.07997	11.68944	13.58984
Theta_H	4.33759	0.24841	2.29300	2.89149
s.d. Theta_H	2.75625	0.23801	1.49713	1.78067

```
                Theta_S     2.53683     1.15702     1.84693     0.97567
            s.d. Theta_S     1.16531     0.78108     0.97320     0.27170
                Theta_pi     1.75263     0.75000     1.25132     0.70897
            s.d. Theta_pi     1.18556     0.69917     0.94237     0.34393

------------------------------------------------------------------------

==========================================================================
==
== GENETIC STRUCTURE ANALYSIS
==========================================================================
==


Number of usable loci for distance computation : 317
Allowed level of missing data                  : 0.05000

List of usable loci :
---------------------
   1     2     3     4     5     6     7     8     9    10    11    12    13
14    15
  16    17    18    19    20    21    22    23    24    25    26    27    28
29    30
  31    32    33    34    35    36    37    38    39    40    41    42    57
58    59
  60    61    62    63    64    65    66    67    68    69    70    71    72
73    74
  75    76    77    78    79    80    81    82    83    84    85    86   101
102   103
 104   105   106   107   108   109   110   111   112   113   114   115   116
117   118
 119   120   121   122   123   124   125   126   127   128   129   130   131
132   133
 134   135   136   137   138   139   140   141   142   143   144   145   146
147   148
 149   150   151   152   153   154   155   156   157   158   159   160   161
162   163
 164   165   166   167   168   173   174   175   176   177   178   179   180
181   182
 183   184   185   186   187   188   189   190   191   192   193   194   195
196   197
 198   199   200   201   202   203   204   205   206   207   208   209   210
211   212
 213   214   215   216   217   218   219   220   221   222   223   224   225
226   227
 228   229   230   231   232   233   234   235   236   237   238   239   240
241   242
 243   244   245   246   247   248   249   250   251   252   253   254   255
256   257
 258   259   260   261   262   263   264   265   266   267   268   269   270
271   272
 273   274   275   276   277   278   279   280   281   282   283   284   285
286   287
 288   289   290   291   292   293   294   295   296   297   298   299   300
301   302
 303   304   305   306   307   308   309   310   311   312   313   314   315
316   317
```

```
 318   319   320   321   322   323   324   325   326   327   328   329   330
331   332
 333   334   335   336   337   338   339   340   341   342   343   344   345
346   347
 348   349

List of loci with too much missing data :
-----------------------------------------

  43    44    45    46    47    48    49    50    51    52    53    54    55
56    87
  88    89    90    91    92    93    94    95    96    97    98    99   100
169   170
 171   172




==========================================
MINIMUM SPANNING TREE between       12 OTUs
==========================================
Reference:
Rohlf, F.J., 1973.

   OTU 1     OTU 2    Connection length
   =====     =====    =================
      H1        H2             1.00000
      H2        H4             1.00000
      H1        H5             1.00000
      H4        H6             1.00000
      H4        H7             1.00000
      H6        H8             1.00000
      H5        H9             1.00000
      H9       H10             1.00000
      H9       H11             1.00000
      H4       H12             1.00000
      H2        H3             2.00000

------------------------------------------
NEXUS notation for MST
------------------------------------------

#NEXUS
begin trees;  [NEXUS Treefile section generated by Arlequin]
tree MST_AMOVA_MST = [&U] ((H1:0, (H5:0, ((H9:0, H11:1.00000):0,
H10:1.00000):1.00000):1.00000):0, ((H2:0, H3:2.00000):0, (((H4:0,
H12:1.00000):0, H7:1.00000):0, (H6:0, H8:1.00000):1.00000):1.00000):1.00000);
end;




=====================================================================
Alternative connections between OTUs
to extend the minimum spanning tree into a MINIMUM SPANNING NETWORK
=====================================================================
```

```
     OTU    List of alternative links
     ===    =========================
      H4       H5 (1.00000)



=====================================================================
  AMOVA ANALYSIS
=====================================================================

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Computing conventional F-Statistics from haplotype frequencies

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AMOVA design and results :
-------------------------


Weir, B.S. and Cockerham, C.C. 1984.
Excoffier, L., Smouse, P., and Quattro, J. 1992.
Weir, B. S., 1996.
-----------------------------------------------------------------------
 Source of                 Sum of      Variance         Percentage
 variation      d.f.       squares     components       of variation
-----------------------------------------------------------------------
 Among
 populations      1          0.804        0.04044 Va            10.59

 Within
 populations     26          8.875        0.34135 Vb            89.41
-----------------------------------------------------------------------
 Total           27          9.679        0.38179
-----------------------------------------------------------------------
 Fixation Index      FST :      0.10593
-----------------------------------------------------------------------

Significance tests (1023 permutations)
------------------

Va and FST : P(rand. value > obs. value)  =  0.03421
             P(rand. value = obs. value)  =  0.01564
                                P-value =  0.04985+-0.00572


---------------------------
Genetic structure to test :
---------------------------

No. of Groups = 1


        [[Structure]]

                StructureName = "New Edited Structure"
```

```
               NbGroups = 1
        #Group1
               Group={
               "Luci"
               "Sept"
               }

---------------------------


Distance method: Pairwise difference

-------------------------
AMOVA design and results :
-------------------------

Weir, B.S. and Cockerham, C.C. 1984.
Excoffier, L., Smouse, P., and Quattro, J. 1992.
Weir, B. S., 1996.
-------------------------------------------------------------------------
 Source of                  Sum of      Variance         Percentage
 variation      d.f.        squares     components       of variation
-------------------------------------------------------------------------
 Among
 populations      1          0.546       -0.01706 Va            -2.35

 Within
 populations     26         19.275        0.74135 Vb           102.35
-------------------------------------------------------------------------
 Total           27         19.821        0.72429
-------------------------------------------------------------------------
 Fixation Index      FST :     -0.02355
-------------------------------------------------------------------------

Significance tests (1023 permutations)
------------------

Va and FST : P(rand. value > obs. value)  =  0.59433
             P(rand. value = obs. value)  =  0.04106
                                 P-value =  0.63539+-0.01284
```

Table 2. Inter-haplotypic distance matrix computed from combined data.

Inter-haplotypic distance matrix (s.d. above diagonal):

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12
H1		0.9984	1.7238	1.4097	0.9984	1.7238	1.7238	1.9873	1.4097	1.7238	1.7238	1.7238
H2	1.0000		1.4097	0.9984	1.4097	1.4097	1.4097	1.7238	1.7238	1.9873	1.9873	1.4097
H3	3.0000	2.0000		1.7238	1.9873	1.9873	1.9873	2.2184	2.2184	2.4262	2.4262	1.9873
H4	2.0000	1.0000	3.0000		0.9984	0.9984	0.9984	1.4097	1.4097	1.7238	1.7238	0.9984
H5	1.0000	2.0000	4.0000	1.0000		1.4097	1.4097	1.7238	0.9984	1.4097	1.4097	1.4097
H6	3.0000	2.0000	4.0000	1.0000	2.0000		1.4097	0.9984	1.7238	1.9873	1.9873	1.4097
H7	3.0000	2.0000	4.0000	1.0000	2.0000	2.0000		1.7238	1.7238	1.9873	1.9873	1.4097
H8	4.0000	3.0000	5.0000	2.0000	3.0000	1.0000	3.0000		1.9873	2.2184	2.2184	1.7238
H9	2.0000	3.0000	5.0000	2.0000	1.0000	3.0000	3.0000	4.0000		0.9984	0.9984	1.4097
H10	3.0000	4.0000	6.0000	3.0000	2.0000	4.0000	4.0000	5.0000	1.0000		1.4097	1.7238
H11	3.0000	4.0000	6.0000	3.0000	2.0000	4.0000	4.0000	5.0000	1.0000	2.0000		1.7238
H12	3.0000	2.0000	4.0000	1.0000	2.0000	2.0000	2.0000	3.0000	2.0000	3.0000	3.0000	