BACTERIA INVOLVED IN THE HEALTH OF HONEY BEE (*APIS MELLIFERA*) COLONIES

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

In recent years there has been a dramatic worldwide increase in the loss of honey bee colonies. The actual winter loss of honey bees in the United States has been reported to be ~30%, which is double the historic level (1). Honey bee population decline has received much media attention due to the important ecosystem service they provide; pollination. Honey bees pollinate over 90 commercial crops making them important in agriculture and to the economy (2). Causes of pollinator declines are linked to pests and pathogens, pesticide exposure, land use changes and losses in nutritional foraging resources. The bacterial flora in colonies protects the honey bees from pathogens by preventing their colonization (2). Production of antimicrobial compounds such as peptide antibiotics by members of the flora is thought to prevent pathogen growth (3, 4). The micro flora has also been found to benefit the honey bee colony by aiding in the conversion of pollen to bee bread, a protein rich food, by altering the vitamin content, pH, amino acid profile, and the amount of complex polysaccharides (2). These findings suggest that the bacterial flora in bee bread is important for honey bee colony health.

Oxytetracycline HCl, Streptomycin, Tylosin Tartrate, and Fumagillin-B are antibiotics used in honey bee colonies and on plants pollinated by honey bees (5-11). These antibiotics prevent or control pathogens such as *Paenibacillus larvae*, *Melissococcus plutonius*, and *Nosema apis*, which cause American Foulbrood,

European Foulbrood, and the Nosema disease respectively (11, 12). However, the mode of action of these antibiotics is not specific towards pathogens; therefore commensal organisms can also be affected.

The goal of this research was to determine if the bacterial flora of bee bread in honey bee colonies is a reflection of the health of the colony. Bee bread was sampled from 15 colonies in the University of Delaware South Campus Research Apiary once a month from May to October 2013. In collaboration with Dr. Deborah Delaney's lab (University of Delaware Department of Entomology and Wild Life Ecology), colony characteristics including total number of bees, total number of brood, brood pattern, and the amounts of honey, pollen, and nectar were also determined. Polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE) were used in combination as a bacterial fingerprinting technique to get a profile of all the culturable and nonculturable bacteria present. Culturable bacteria were isolated from the bee bread samples and identified using fatty acid methyl ester (FAME) analysis. Bacterial isolates from bee bread were also tested for susceptibility to Oxytetracycline HCl, Streptomycin, Tylosin Tartrate, Fumagillin-B, and Ampicillin to determine their effect.

FAME analysis identified some of the same core organisms reported by other researchers as well as diverse organisms, which may be environmental bacteria transferred into the colony. PCR-DGGE revealed that bacterial flora of bee bread changed as the season progressed. Principal component analysis was done on the bee

bread bacterial banding patterns produced by PCR-DGGE which suggested that the bacterial flora of bee bread is one of multiple factors involved in the survival of honey bee colonies after the winter season. Some *Enterobacteriaceae* were susceptible to Oxytetracycline HCl and Streptomycin, which suggests that their roles in the colony should be further researched.

Chapter 1

INTRODUCTION

1.1 *Apis mellifera*

Apis mellifera L., commonly called the 'Western' honey bee, are social insects that are managed globally for agricultural use (13). Honey bees pollinate over 90 commercial crops in the United States, adding $15 billion in value to agricultural crops each year (14). Globally, honey bees are beneficial for agriculture and the economy because they are inexpensive, can easily be maintained and easily transported for pollination (13). Though they are not the only pollinators of animal-dependent plant pollination, they are one of the most important pollinators of crop monocultures (13).

The stability of the honey bee colony depends on three types of adult bees, the queen, worker, and drone. The drone develops from an unfertilized egg. The worker bee and queen both develop from fertilized eggs, and their developmental pathway is determined based on the quality and quantity of food the larvae are fed (15). The queen, a female adult bee, lays eggs and produces chemicals that help maintain colony cohesion and regulate colony reproduction (16). Workers are also female. They differ from queen bees by having underdeveloped reproductive organs and are smaller in size (16). They maintain the colony by foraging, defending the colony, brood rearing, and construction (15). Drones, male adult bees, have the single purpose of mating with virgin queens. Queens, workers, and drones undergo four stages of development which average 16, 21, and 24 days respectively (16). Due to this short development

time along with the queen's ability to produce 1,500 eggs per day, the honey bee colony can have as few as 10,000 bees in February and March and as many as 60,000 honey bees in June making managed honey bee colonies very valuable to agriculture and the economy (16).

1.2 Honey Bee Decline

Since honey bees have an important role in agriculture and the economy, their recent decline is of concern. Colony Collapse Disorder (CCD) was a term introduced in 2006 until 2011 to describe the symptoms associated with the sudden loss of honey bee colonies, and specifically characterized as a colony with only the queen and a few workers remaining (17). The affected colony was also avoided by other bees in the area (17). Many factors have been associated with CCD such as pathogens and parasites, environmental and management stressors, and poor nutrition; however, no one cause or group of factors has been shown to cause the disorder (17). Research has shown some factors could be predictive markers of CCD such as increased levels of *Varroa destructor*, *Nosema ceranae*, and Deformed Wing Virus (DWV), as well as decreased levels of vitellogenin (18). However, no cause has been definitively identified.

Honey bee declines have also occurred before and after CCD. In the 1980s the introduction of parasites such as the ectoparasitic mite *Varroa destructor* increased the occurrence of certain viruses which led to colony decline (19, 20). In the winter of 2011-2012 the annual winter loss of honey bees was 22%, which was 10% less than the honey bee winter loss from 2006 to 2010. However, the winter of 2012-2013 had an average winter loss of 31.1% (21). This history of decline makes having a predictive system for colony survivorship beneficial to beekeepers.

1.3 Bacteria Associated with *Apis mellifera*

Microbial niches exist in the honey bee gut, in their nutrients, and in the colony itself (3). Through culture dependent methods honey bees have been found generally free of microorganisms from eggs to emergence as adults (22). An exception of this occurs when microorganisms are acquired such as by ingestion of contaminated food and are then usually removed by defecation toward the end of the larval feeding period (22). Adult honey bees acquire an intestinal microbial flora within four days after emerging through food exchange with other bees and through the consumption of bee bread (22).

The honey bee micro flora is important as many host-symbiont interactions occur in the colony (3). Microbial species are thought to have coevolved in mutual dependence with social insect species, such *Apis mellifera*. This coevolution allows microbes to survive in a niche with available nutrients, while the honey bees benefit from the valuable roles the bacteria play in the colony (23, 24). These benefits include protecting honey bees from pathogens, aiding in the digestion of food, and helping in the fermentation processes, which are necessary for bee bread and honey production (2, 25). Microorganisms have also been reported to benefit honey bees by producing or concentrating vitamins, essential amino acids, and sterols or by utilizing waste compounds (26). Benefits such as these are also seen in bee bread and other honey bee nutrients where an increase in vitamins and specific amino acids has been reported (27, 28).

Both commensal and pathogenic microorganisms have been shown to affect the health of the colony. *Paenibacillus larvae* and *Melissococcus plutonius* are two bacteria that cause American foulbrood disease and European foulbrood disease respectively (29). These diseases damage the colony by infecting honey bee larvae

ultimately leading to colony loss. Though *P. larvae* and *M. plutonius* are most commonly associated with bacterial infections, other bacterial pathogens exist in the colony including *Spirolasma apis* and *Spiroplasma melliferum*, *Achromobacter euridice*, *Anterococcus faecalis*, *Paenibacillus alvei*, and *Brevivacillus lateosporus* (29). *Spiroplasma* infections cause death in the honey bee when these organisms get through the gut barrier and invade the hemolymph (29, 30). All of the latter are secondary invaders of European Foulbrood and contribute to the odor of the dead bee brood (30). *Pseudomonas aeruginosa*, additionally is a honey bee pathogen and causes septicemia in adult honey bees (30). As with the human body's micro flora, the micro flora of honey bees prevents colonization of competing pathogens (31). This protective role of microbes makes the bacterial flora of honey bees an important area of study.

1.4 Bee Bread

Pollen serves as a source of vitamins, proteins, fatty acids, lipids, sterols, minerals, and carbohydrates (15, 28, 32, 33). It is converted to bee bread after being mixed with nectar and secretions from the bee's salivary glands (23, 33). Introduced microorganisms such as bacteria and yeast are thought to perform lactic acid fermentation, which results in the conversion of pollen to bee bread (23, 28).

The mixing of foregut contents with newly collected pollen results in the initial introduction of microbes into the pollen which is then stored on the corbiculae (23, 34, 35). The corbicular pollen is then packed into the cells of the colony and sealed with honey during which more microorganisms are introduced (23, 36). Microbial activity results in a lowering of the pH of the pollen mixture and a change in the chemical composition (23, 28, 33). The resulting bee bread differs from pollen by having a

higher acidity due to lactic acid, large amounts of vitamin K, and increased concentrations of amino acids such as alanine, aspartic acid, glutamine, leucine, threonine, and valine (23, 27, 28). Protein concentrations have been found to be lower in bee bread samples compared to pollen and concentrations of the amino acids methionine, tryptophan, and proline were also lower (28). Microbial interactions resulting in amino acid synthesis or deamination and hydrolysis have been proposed as a reason for the variations in these protein and amino acid concentrations (28).

Spoilage of bee bread is also prevented by microorganisms (2, 23). Recent research suggests that Lactic Acid Bacteria are important for the production and storage of bee bread as well as for defense against pathogens (23). Since bacteria from the honey bee stomach, the colony, and the environment are used in the conversion of pollen to bee bread, microbial analysis of this material may provide insight into the beneficial bacterial flora of the colony. This information can be obtained without the need to sacrifice bees.

1.5 Antibiotics

Antibiotics have historically been used on both bees and plants pollinated by bees as early as the 1950s (5, 37, 38). To date, Oxytetracycline Hydrochloride, Tylosin Tartrate, and Fumagilin-B are FDA approved for use on honeybees and Oxytetracycline and Streptomycin are FDA approved for plants pollinated by honey bees (5-9, 11, 37, 38). Since antibiotics affect both targeted pathogens and commensal bacteria, organisms isolated in this study were tested for susceptibility to these antibiotics along with Ampicillin, an antibiotic that is commonly used worldwide (6, 10).

1.5.1 Oxytetracycline Hydrochloride

P. larvae and *M. plutonious* are two honey bee pathogens that cause American Foulbrood and European Foulbrood respectively (12). Oxytetracycline Hydrocloride (Terramycin®), a tetracycline analog isolated from the actinomycete *Streptomyces rimosus*, was found to control American Foulbrood in 1951 (37, 39). European Foulbrood has also been shown to be controlled by Oxytetracycline Hydrochloride (12). Oxytetracycline Hydrochloride affects a wide range of Gram-negative and Gram-positive bacteria (40). Its action is bacteriostatic. It inhibits bacterial protein synthesis by binding to the 30S ribosomal subunit, which prevents the aminoacyl-tRNA from binding to the ribosome (40-42).

Along with Oxytetracycline's role in controlling American Foulbrood and European Foulbrood, it is also one of two antibiotics registered by the US Environmental Protection Agency (EPA) for plant agriculture (43). Oxytetracycline is used in the prevention of plant bacterial diseases including fire blight, which is a major plant disease caused by *Erwinia amylovora* (43). Crops that Oxytetracycline is sprayed on specifically include apples, nectarine, peaches, pears, and sugar beets (43). It was recently found that some honey bee gut bacteria are resistant to Oxytetracycline Hydrochloride. In this study, resistance genes were found ubiquitously across samples, but were not found in honey bee colonies that were not exposed to Oxytetracycline (38). This suggested that the long-term treatment of Oxytetracycline caused the accumulation of resistance genes from mobile resistance loci from pathogens and agricultural sites (38). Therefore, determining the antibiotic susceptibility of bacteria found in bee bread to Oxytetracycline and other antibiotics used in the honey bee colony or on plants pollinated by honey bees will also be beneficial in this area of study.

1.5.2 Streptomycin

Streptomycin is an aminoglycoside produced by the soil actinomycete *Streptomyces griseus* (7). It was registered in 1955 for use in controlling bacterial and fungal diseases in agricultural and non-agricultural crops, and is the second of two antibiotics registered for use by the EPA in agriculture (7, 43). Streptomycin irreversibly binds to16S rRNA and to the 30S ribosomal subunit in proteins. Its binding leads to interference of the initiation complex by interfering with the decoding site on 16S rRNA, which results in the mRNA misreading (44, 45). This interference leads to incorrect amino acid insertion, which alters or disables the peptide's function (44, 45). Though Streptomycin also has many clinical uses, 58% of its total use is for the control of fire blight in crops (7). These crops include apples, beans, celery, cranapples, pears, peppers, potatoes, quince, tomatoes, sugar beets, and tobacco (43). Streptomycin residues are reported to be non-detectable (<0.5ppm) in or on crops when treated according to label directions, however because overuse of antibiotics such as streptomycin can lead to resistance, it would be useful to determine the antibiotic susceptibility of bacteria found in bee bread (46).

1.5.3 Tylosin Tartrate

Tylosin is a natural macrolide produced by *Streptomyces fradiae* (47). Similarly to other macrolides, Tylosin is chemically altered in order to increase its activity against some resistant organisms and to improve its pharmacokinetic profile (47). Hitchcock et al. found in 1969 that Tylosin Tartrate could control American Foulbrood disease, however it was not approved for use until 2005 (5, 8, 9, 37). Though Tylosin's exact mode of action is not completely understood, antibiotics in the macrolide class inhibit bacterial protein synthesis by binding to the L27 protein of the

50S ribosomal subunit (48). This binding inhibits translocation of the peptidyl-tRNA from the acceptor to the donor site of the ribosome (48). Tylosin is more effective in crossing the cell membrane of Gram-positive bacteria; however some Gram-negative bacteria are still affected by Tylosin (48, 49).

1.5.4 Bicyclohexylammonium Fumagillin (Fumagillin-B)

Bicyclohexylammonium Fumagillin (Fumagillin-B) is an antibiotic isolated from the fungus *Aspergillus fumigatus* (11). It is the only antibiotic approved for treatment of *Nosema apis* and *Nosema ceranae*, both of which cause Nosemosis (the Nosema Disease) (11). This disease is transmitted by ingestion of spores from the environment. It can lead to a decreased worker lifespan, a weakening of colonies, and can ultimately lead to weakened colonies and economic damage (13, 50). Fumagillin affects microspordia survival by irreversibly inhibiting the enzyme aminopeptidase-2 (MetAP2), which plays a role in post-translational modification of proteins (11, 51). Though Fumagillin has been used on honey bees for over 50 years, it was recently reported that the recommended doses of Fumagillin affect bee physiology at concentrations that no longer suppress *N. ceranae,* which suggests new treatments for Nosema are necessary (11). Though this antibiotic's mode of action is not reported to affect bacteria, it would be of interest to compare antibiotic susceptibility results from all antibiotics that bees are exposed to.

1.5.5 Ampicillin

Ampicillin has been used in the United States since the 1960s and is one of the most commonly used antibiotics worldwide (6). Ampicillin is an antibiotic in the Aminopenicillin family, a group also known as third generation penicillins (6).

Antibiotics in this group undergo semisynthetic modifications, which give them a broader spectrum of activity than natural penicillin (52). They bind to bacterial proteins and inhibit synthesis of the bacterial cell wall, which causes cell lysis (52). Ampicillin is not used on honey bees or on crops pollinated by honey bees, however, because it is a commonly used antibiotic it would be beneficial to observe the antibiotic susceptibility of bacteria found in bee bread to Ampicillin.

1.6 Hypotheses

The goal of this project is to create a diagnostic tool to predict the declination of healthy honey bee colonies. Since the bacterial flora of honey bees plays many important roles that affect the health of the colony, it may be possible to use the molecular fingerprint technique, Polymerase Chain Reaction – Denaturing Gradient Gel Electrophoresis (PCR-DGGE), to track the bacterial flora of bee bread. Further analysis of the banding patterns from PCR-DGGE may determine if there are statistical differences between bee bread samples from healthy colonies and colonies that are declining. A second goal of this project is to identify the bacteria found in bee bread by FAME Analysis and the WalkAway 40®. This may provide further insight on bacteria associated with the honey bee colonies. Antibiotics can inhibit the growth of both targeted pathogens and other bacteria that are affected by an antibiotic's mechanism of action. Therefore, a third goal of this project is to test bacteria isolated in this study for antibiotic susceptibility to antibiotics used in honey bee colonies and on plants pollinated by honey bees.

The two hypotheses of this project are (1) The state of a honey bee colony can be tracked by observing the bacterial flora of bee bread and (2) the widespread use of

antibiotics in honey bee colonies can lead to the resistance of organisms to antibiotics used in the colony.

Chapter 2

MATERIALS AND METHODS

2.1 Strains

2.1.1 Bee Bread Sources and Sampling

Bee bread was sampled from thirty two colonies in the University of Delaware South Campus Research Apiary once per month from May to October 2013 (Table 1). To carry out the sampling 200 µl pipette tips were placed onto the bee bread and gently rotated until the bee bread filled approximately 2 cm up the tip. A variety of bee bread was sampled including light, dark, gloss, matte, soft and sticky. Three to five samples were taken from a given colony per sampling. All samples were stored at 4^{o}C until further processing.

2.1.2 Bacterial Isolation from Bee Bread

Fifteen of the thirty two bee bread samples were inoculated onto microbiological media for further testing (Table 1). Bee bread in one 200 µl tip was extruded with a sterile inoculating needle into a 1.5 ml Eppendorf tube containing 600µl of EPA dilution water. It was gently mixed with a 200 µl pipette man, and vortexed after which 100 µl of the bee bread-EPA dilution water solution was spread onto media containing plates. Plates were incubated at 28^{o}C for 48 hours or until substantial bacterial growth appeared.

2.1.3 Bacterial Isolation

Morphologically unique individual colonies from each sampling were restreaked onto Luria-Bertani (LB) agar plates. Individual colonies from these plates were then consecutively inoculated onto LB plates twice to ensure purity of each isolate. Isolates were stored at 4^{o}C until testing and were then frozen at -80^{o}C after identification by FAME analysis.

2.2 Media

2.2.1 Agar Supplemented Media

Tryptic Soy Agar (TSA) and Luria-Bertani Agar (LB) were used for the isolation and cultivation of bacteria from bee bread. These standard media were amended to determine which medium/amendment combination cultivated the largest variety of bacteria. The TSA was amended with pollen or pollen and yeast, and the LB was amended with pollen.

2.2.2 Gellan Gum Supplemented Media

LB, LB modified with Gellan Gum and Minimal Salts Media modified with Gellan gum were tested to determine if use of any of these media resulted in an increase in the diversity and quantity of microorganisms isolated. This increase was observed by Tamaki et al., 2005 when freshwater lakes were sampled for bacteria (53). All media with Gellan Gum also contained 0.03 grams of sodium pyruvate to degrade hydrogen peroxide generated through autoclaving.

2.2.3 Low Nutrient Media

Nutrient Agar (NA) and R2A Agar were used as low nutrient media for the isolation of slow growing organisms. These media were compared to see which medium supported the growth of a larger variety of bacteria.

2.3 DNA Isolation

2.3.1 Bee Bread DNA Isolation

The MoBIO PowerPlant® Pro DNA Isolation Kit was used to isolate DNA from bee bread samples (MoBio Laboratories, Inc.). Individual bee bread samples from 200 µl Eppendorf pipette tips were introduced into 2 ml PowerPlant® Bead Tubes. The protocol was then carried out according to the manufacturer's instructions with the modification of doubled centrifugation times to ensure removal of impurities. The DNA concentration, 260/280 ratio, and 260/230 ratio were determined using a NanoDrop 1000 Spectrophotometer (ThermoScientific).

2.3.2 Bacterial DNA Isolation

The Promega Wizard® Genomic DNA Purification Kit was used to isolate DNA from organisms used in the denaturing gradient gel electrophoresis (DGGE) ladder (Promega Corporation). The DGGE ladder was prepared using equal quantities of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Micrococcus luteus* (Columbia). These organisms provided markers covering a range of GC content of approximately 32.9%, 50.6%, 66.6%, and 73% respectively (54). For DNA isolation, a visible number of colonies of each isolate were placed in a 1.5 mL Eppendorf tube with 480 µl of 50 mM EDTA and 120 µl of lysozyme. The protocol was carried out according to the manufacturer's direction with the

modifications of 5µl of RNase Solution used per sample and repeating the use and aspirating of ethanol. DNA concentrations, 260/280 ratio, and 260/230 ratio were tested on a NanoDrop 1000 Spectrophotometer.

2.3.3 Bacterial DNA Ethanol Precipitation

An ethanol precipitation was done on each DGGE ladder organism to concentrate and clean the DNA. Nine µl of 4^{o}C 3M sodium acetate and 200 µl of 4^{o}C 100% ethanol were added to each 1.5 ml tube of DNA. The tubes were mixed by gentle inversion and incubated at -20^{o}C overnight. They were then centrifuged for 15 minutes at 12,000 x g in a microcentrifuge at 4^{o}C. The supernatant was removed and 500 µl of 4^{o}C 70% ethanol was added. Each tube was then inverted to mix and centrifuged for 5 minutes at 12,000 x g. The precipitated DNA was then dissolved in 75 µl of DNA rehydration solution (Promega). Fifteen to twenty µl of a stock of equal quantities of ethanol precipitated DNA from *S. aureus*, *P. aeruginosa*, *E. coli*, and *M. luteus* were used for all gels.

2.4 Denaturing Gradient Gel Electrophoresis

2.4.1 Polymerase Chain Reaction (PCR) of 16S rRNA Gene

The 16S rRNA gene from the DNA of bee bread and bacterial isolates was amplified using the primers 318F-GC, which had a 39 base-pair GC clamp attached to the 3' end and 518R. The 16S rRNA gene was chosen for amplification because it is universal to bacteria. The primer sequence for the 318F primer was 5'- CCT ACG GGA GGC AGC AG -3'. The sequence of the GC Clamp added to the 3' end of the forward primer was 5'-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG- 3'. The primer sequence of the 518R primer was 5'-ATT ACC

GCG GCT GCT GG-3'. All PCRs were performed on a PTC-100TM Programmable Thermal Controller (MJ Research, Inc.). The PCR mixtures contained 5 or 10 µl of DNA and 0 or 10 µl of nuclease free water for DNA samples with greater than 10ng/µl of DNA or less than 10 ng/µl of DNA respectively. PCR mixtures also included 1.25 µl of each primer and 12.5 µl of master mix (Promega). The total reaction volumes were 25 µl. DGGE ladder organisms had total reaction volumes of 50 µl each with twice the volume of each component. All organisms used for the DGGE ladder were amplified in quadruplicate during the same PCR cycle. Each organism was then added in equal quantities to a 1.5 ml tube and stored at 4^{o}C as a stock. Touchdown PCR was used to amplify the 16S rRNA by lowering the annealing temperature over a series of cycles to increase the specificity of the reaction (55, 56). The temperature programs for Touchdown PCR were 94^{o}C for 1 minute, followed by 3 cycles of 15 seconds at 94^{o}C, 15 seconds at 61^{o}C, and 1 minute at 72^{o}C. The following phase included 3 cycles of 15 seconds at 94^{o}C, 15 seconds at 58^{o}C, and 1 minute at 72^{o}C. The third phase included 28 cycles of 15 seconds at 94^{o}C, 15 seconds at 55^{o}C, and 1 minute at 72^{o}C. Bee bread from colonies 3-August, 11-September, 11-October, s12-September, s12-October, 13-October, 14-October, 16-October, 19-September, 20-October, and 21-October underwent three additional cycles of the third phase to ensure ample DNA was present for denaturing gradient gel electrophoresis. All samples of bee bread DNA were verified on a 2% agarose gel prior to denaturing gradient gel electrophoresis (DGGE). Only bee bread DNA samples with visual PCR products by agarose gel electrophoresis were used for DGGE.

2.4.2 Denaturing Gradient Gel Electrophoresis

PCR-DGGE of the 16S rRNA gene of bee bread was performed using a DCodeTM system (Bio-Rad Laboratories). Twenty µl of PCR products from bee bread and the DGGE ladder were loaded onto a 6% polyacrylamide gel in 1 X TAE at 60^{o}C (Thermo Scientific). The polyacrylamide gel was made with 0% denaturing solution (15 ml 40% acrylamide/bis, 2ml 50 X TAE, 83 ml of double distilled water) and a 100% denaturing solution (15ml ml acrylamide/bis, 2ml 50 X TAE, 40ml deionized formamide, 42 grams of urea, to 100ml of double distilled water) for a 30% (top) to 70% (bottom) denaturing gradient produced by increasing concentrations of urea and formamide. Ten µl of N,N,N′,N′-Tetramethylethylenediamine (TEMED) and 120 µl of 10% Ammonium Persulfate (APS) were used to polymerize the gel. Approximately 2 ml of water-saturated butanol was immediately added to the top of the gel and left to polymerize for 2 hours. After removing the water-saturated butanol 5 ml of 0% denaturing solution with 10 µl of TEMED and 150 µl of APS was added to the top of the gel and left to polymerize for 2 hours. The electrophoresis was run for approximately 15 hours at 60^{o}C and 60V. After electrophoresis the gels were stained in ethidium bromide (10 mg/µl) for 30 minutes then photographed on an Alpha Imager® HP System. DGGE profiles were analyzed using the GelCompar II ® software program (Applied Maths).

2.5 Fatty Acid Methyl Ester (FAME) Analysis for Identification of Aerobes and Actinomycetes

All bacteria were subjected to FAME analysis using the Sherlock Microbial Identification System according to the manufacturer's instructions (MIDI, Inc.) (57). Each bacterial isolate was restreaked onto Tryptic Soy Broth Agar (TSBA) (equivalent to TSA) for 24 hours at 28^{o}C or until the culture reached late-log phase indicated by

confluent growth of colonies (57). The bacteria were harvested into glass culture tubes (Corning™, Pyrex™) and frozen at -20°C until used for FAME analysis.

For cultivation of actinomycetes, approximately 3 colonies of each isolate were inoculated into 20µl of Trypticase Soy Broth (TSB) in a cotton-stopped Erlenmeyer flask. The flasks were then secured and shaken at 150 RPM for at least 48 hours at 28°C or until over twice the cell mass was obtained (Controlled Environment Incubator Shaker- New Brunswick Scientific Co. Inc. / Eppendorf). The bacterial colonies were then harvested by vacuum filtration onto 0.45 µm cellulose filters then scraped into glass culture tubes and stored at -20°C as with the aerobes.

In preparation for analysis 1.0 ml of a solution of sodium hydroxide and methanol (45 grams Sodium Hydroxide (certified ACS), 150 ml Methanol (HPLC grade), 150 ml deionized distilled water) was added to each culture tube containing the harvested aerobic or actinomycete bacteria. The culture tubes were then vortexed before inserting in a hot water bath (100°C) for five minutes. After a second vortexing the culture tubes were placed back into the hot water bath for twenty five minutes. Two ml of a solution of 6N hydrochloric acid and methanol (325 ml 6.00N Hydrochloride and 275 ml Methanol (HPLC grade) was then added to each tube. The tubes were vortexed briefly before being placed into an 80°C water bath for ten minutes. 1.25 ml of a hexane and methyl tert-butyl ether solution was added to each tube and each tube was inverted for ten minutes (200 ml Hexane (HPLC Grade) and 200 ml Methyl tert-butyl ether (HPLC Grade). All samples were then washed in sodium hydroxide and inverted for 5 minutes (10.8 grams Sodium hydroxide (certified ACS) and 900ml deionized distilled water). The aqueous phase was then removed and 3.0 ml of sodium hydroxide was added to each tube. Two thirds of the organic phase

was then pipetted into a GC vial for FAME analysis. FAME analysis was carried out with an Agilent 6890N Gas Chromatagraph using an Agilent 19091B-102 Ultra 2 capillary GC column. FAME analysis was carried out using a FID Detection Flame Ionization Detector with a flow rate of 1 ml/min. Bacterial isolates were identified on the MIDI Sherlock® Microbial Identification System using the TSBA6 library for anaerobes and ACTIN1 library for actinomycetes. FAME Analysis results were analyzed using the Canoco software program.

2.6 WalkAway®40 System for Identification of *Enterobacteriaceae*

The WalkAway® 40 was used to identify organisms of the family *Enterobacteriaceae* (Siemens Corporation). Isolates were streaked onto Columbia CNA plates and MacConkey agar plates to verify the organisms were Gram-negative. The organisms were then restreaked onto blood agar plates and incubated at 28^{o}C for 24 hours. Beta hemolysis ability was then reported for each organism. Oxidase tests were done on all organisms to test for the presence of cytochrome oxidase (or for the production of indophenol oxidase) using Oxidase Reagent Droppers, which contained a 1% aqueous solution of N,N,N',N'-tetramethyl-p-phenylenediamine-dihydrochloride (Becton Dickinson and Company). Organisms were inoculated into 3 ml tubes of Inoculum Water until a McFarland Standard of 1.0 was reached. After vortexing, 100 µl of each organism was then pipetted into a 25 ml tube of Inoculum Water with PLURONIC®. The tubes of Inoculum Water with PLURONIC® were then poured into seed trays, which were used to dispense 115 µl of the inoculated Inoculum Water with PLURONIC® into the wells of a Negative/Urine Combo 46 Panel. The panels were incubated in a WalkAway® System for 24 hours allowing for the growth and identification of the organisms.

2.7 Antibiotic Susceptibility Testing

Oxytetracycline Hydrochloride, Tylosin Tartrate, Streptomycin, Fumagillin-B, and Ampicillin were used to test for antibiotic susceptibility in thirteen isolates from the June sampling period. The thirteen isolates tested included five *Enterobacteriaceae*, two *Bacillus*, two organisms that were not identified because their Sim index was too low, and one organism that was not identified because there was no match in the library. Antibiotic disks containing 250 µg, 25 µg, 2.5 µg, 0.25 µg, and 0.025 µg were made by pipetting 5 µl of a 50 µg/µl, 5 µg/µl, 0.5 µg/µl, 0.05 µg/µl, and a 0.005 µg/µl solution of each antibiotic onto a sterile 6 mm filter paper disk. All antibiotic concentrations were made in sterile double distilled water except for Fumagillin-B which is immiscible to water and was made in ethanol. Controls were prepared by pipetting 5 µl of a 0 µg/µl solution of each antibiotic onto the sterile 6 mm filter paper disks. After drying disks containing the six concentrations of a given antibiotic they were placed onto a TSA plate previously inoculated with either test or control organisms. *Staphylococcus aureus* and *Escherichia coli* served as the controls. The plates were incubated over night at 28^{o}C then the zone of inhibition was measured as the diameter surrounding the disk where no growth was present on the plate.

The six concentrations and their respective zones of inhibition were then compared to standard concentrations to report zones sizes that indicate resistance, intermediate resistance, or susceptibility (58). Of the thirteen organisms tested for antibiotic susceptibility conclusions were made for *Enterobacteriaceae* since these were the only organisms tested that have reported zone of inhibition sizes indicating resistance or susceptibility (58). Oxytetracycline at a disk potency of 25 µg was compared to the reported standard disk potency of 30 µg. The Streptomycin disk potencies of 2.5 µg and 25 µg used in this study were compared to the standard of 10

µg. Ampicillin disk potencies of 2.5 µg and 25 µg were also compared to the standard disk potency of 10 µg. Tylosin Tartrate disks were not available for purchase. The disks were therefore prepared based on the levels of antibiotic recommended for bee keepers. The manufacturer recommends mixing 200 mg of Tylosin Tartrate with 20 g of sugar. The zone of inhibition was therefore compared at disk potencies of 2.5 and 25 µg of the antibiotic, levels representative of those found in the colony. Fumagillin-B also does not have a reported standard potency, however the manufacturer's recommended concentration of 25 mg/L calculates to 0.025 µg/µl. Disks were prepared at the same six concentrations and compared to this recommended concentration.

2.8 Statistical Analysis

2.8.1 Denaturing Gradient Gel Electrophoresis

The GelCompar II® software program was used to determine bee bread bacterial community DGGE band positions as the length that the band traveled compared to the DGGE ladder positions after normalization (Applied Maths). Bands were selected based on the band heights observed on the densitometric curve, which was dependent on an 8-Bit optical density (OD) range. Bands with a profiling of less than 5% were not seen on the densitometic curve and bands with a profiling between 5-7% were marked as uncertain by the software program. To standardize the band selection any band that had a representative peak on the densitometric curve was marked certain and used in the analysis. Band classes, which were based on position tolerance and optimization settings, were then used to group bands of the same position across the gels together. Position tolerance determined the maximal shift

allowed between two bands to be considered matching and optimization determined the maximal shift allowed between two banding patterns that can be used to determine the best possible matching. The optimization and position tolerance for the comparison of bacterial profiles was 0.00% and 0.26% respectively.

Principal component analysis (PCA) was done based on the band classes present in each DGGE bacterial profile. Eight colony variables were then analyzed by PCA to determine if any of the variables affected the similarity of the bacterial banding pattern of bee bread. The eight variables tested were the number of bees, number of brood cells, number of mites, percent of total frames full of pollen, percent of total frames full of nectar, percent of total frames full of honey, brood pattern and queen status. The brood pattern was determined based on the overall estimate of brood, eggs and larvae found on the frames. Brood patterns of 3 had minimal empty cells, 1 had scattered or inconsistent brood, eggs, or larvae, and 0 had no brood present. A brood pattern of 2 was an intermediate between a pattern of 1 or 3. The queen status was reported as “queen found” or “queen not found” based on if the queen was found during a monthly survey of each colony. Two additional variables were also analyzed by PCA based on the survival of the honey bee colonies. The survival status each month indicated if the honey bee colony that the bee bread was sampled from was alive, dead, or if it died in the following month (indicated on the PCA plots as “died the following month”) The second variable, survival status after the winter, was based on the survival outcome of the honey bee colonies in February 2014. Bee bread samples each month were marked “dead” if it was from a honey bee colony that died during the winter or “alive” if the bee bread sample was from a colony that survived the winter. Dendrograms, which additionally included the band

intensity in its construction, were also used to verify the results of PCA. Dendrograms were created based on the Dice Coefficient and Unweighted Pair Group Method using Arithmetic Averages (UPGMA).

2.8.2 Fatty Acid Methyl Ester Analysis

Fatty acid methyl esters were identified as matches based on the Similarity Index (SI) indicated by the MIDI Sherlock® Microbial Identification System (MIDI Inc.). The SI is a value between 0 and 1.000 that expresses how closely the fatty acid composition of an unknown compares to the mean fatty acid composition of strains used to create the library entry listed as its match. Fatty acid compositions with a SI of at least 0.600 and a separation from the next match by at least 0.100 were suitable for identification. Fatty acid compositions with an SI of at least 0.600, but with less than a 0.100 separation from the next match were not able to be differentiated without further biochemical testing. Fatty acid compositions with an SI of less than 0.600 were reported as not identified because the Sim Index was too low to ensure accuracy of the identification. PCA was done on all bacterial isolates based on the presence or absence of fatty acids within a given sample using the Canoco 4.5 software program (Biometris, Wageningen University and Research Centre).

Chapter 3

RESULTS

3.1 Bacterial Isolation from Bee Bread

The number of colony forming units of bacteria and molds cultured on TSA, TSA with pollen, TSA with yeast and pollen, LB, and LB with pollen were compared to determine which media cultivated the largest quantity of bacteria. TSA, TSA with pollen, and LB had the highest number of bacteria; however there were no significant differences between media types (Figure 1). LB and LB with pollen had the lowest amounts of molds, but there were similarly no significant differences in the number of molds (Figure 1). Since there were no significant differences between media types, LB was chosen to restreak each isolate for purification.

LB, LB with Gellan Gum, Minimal Salts Media, Nutrient Agar, and R2A were later tested to determine if the use of Gellan Gum or a low nutrient medium for slow growing organisms could increase the diversity and quantity of microorganisms isolated. LB with Gellan Gum and R2A appeared to allow for the most diversity and were chosen for all bacterial isolations from bee bread experiments (data not shown).

3.2 Bee Bread DNA Isolation

Of the 32 total colonies sampled from May to October 2013 fifteen colonies were analyzed by PCR-DGGE (Table 1). Thirteen colonies were sampled from May to October and two colonies (s3B1 and s12B2) that were split early in the season were also included in the study beginning in June. Colony 2A2 died of European Foulbrood

(EFB) in July and was not sampled after June. Colonies s3B1 and 19B2 were sampled in all months except October when both colonies died of unknown causes. Colonies 11 and 16 also died in October, however bee bread samples were still taken in all months and their cause of death was unknown. All samples had measurable DNA, however, some did not have a PCR product when verified on a 2% agarose gel and were not used for DGGE (Table 2).

3.3 DGGE Ladder

The Denaturing Gradient Gel Electrophoresis Ladder composed of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, and *Micrococcus luteus* consistently marked gradient positions across the gel (Figure 1a). The bands in each lane indicated the presence of a 16S rRNA gene, in which the mobility of the DNA was significantly slowed due to increasing concentrations of urea and formamide, which denature the DNA based on GC content. Multiple bands were present when the bacterial profiles of individual organisms were observed. The ladders as well as individual organisms had both prominent and less prominent bands; however, only prominent bands were used for normalization of all gels. *B. subtilis* and *E. coli* DNA both observed at the same position on the gel so *B. subtilis* was not used in future ladders. The ethanol precipitation done on each DGGE ladder organism resulted in a stronger banding pattern compared to the DGGE ladder without an ethanol precipitation step (Figure 1b). The ethanol precipitated DGGE ladder was used on all gels.

3.4 Denaturing Gradient Gel Electrophoresis

All fifteen of the colonies' bacterial banding patterns from May to October were visualized by DGGE, which revealed complex bacterial profiles for most of the samples (Figures 3-8). The majority of the bacterial profiles of the bee bread samples had bands with positions representing a broad range of DNA G+C content; however, variation was seen in the amount of bands observed in each DGGE bacterial profile among the bee bread samples (Figures 3-8).

DGGE banding patterns showed that the bacterial profiles varied by month (Figures 3-8). The number of bands observed each month ranged on average from 11.1-14.8 bands/colony per month (Figure 9). Though no significant differences were seen across the months, the number of bands increased from May to June then decreased from July to August. A slight increase was seen in September and October, which both had an average of 13 bands/colony compared to an average of 11 bands/colony in August. Though the band positions varied greatly across the season, two bands with the lowest DNA G+C content observed on the gels were seen on most DGGE bacterial profiles (Arrows B and C in Figure 5). Common bands were also present in a similar range of DNA G+C content as *M. luteus* on the DGGE Ladder (Arrow A in Figure 5). Variation in the DGGE banding patterns was also observed from colony to colony. Despite coming from the same sampling period, bee bread samples from one colony did not appear similar when compared to another bacterial profile from a different colony in the same month (Figures 3-8).

When PCA was done to compare all bee bread samples the majority of the bee bread samples were grouped together near the origin of the PCA plot with many outliers surrounding the primary group (Appendix A Figure A1). The bee bread bacterial flora was not separated by month when all samples were considered

(Appendix A Figure A1). The number of bees, brood, and mites and the amount of pollen, nectar, and honey was compared to the bee bread bacterial profiles by PCA, but the grouping on the PCA plot did not reflect these variables (Appendix A-G Figures A1 A, B1 A,C1 A, D1 A, E1 A, F1 A, and G1 A). The brood pattern, queen status, monthly survival status, and winter survival status also did not explain the grouping of all the bee bread samples (Appendix H-J Figures H1 A, I1 A, and J1 A). The dendrogram similarly to the PCA plot did not explain the grouping of bee bread samples by the winter survival status (Figure 11 A).

Groups were also observed when the bee bread samples were compared by PCA by month. When only May bee bread samples were compared by PCA two groups and two outliers were observed (Appendix B1 Figure B1 B). These groups did not differ by the amount of brood, mites, pollen, nectar or honey, nor did the groups differ by brood pattern (Appendix C-G Figures C1 B, D1 B, E1 B, F1 B, and G1B). The number of bees, the queen status, and the monthly survival status were unable to explain the variabion because all colonies in May had less than 10,001 bees, a queen present, and were alive in May as well as in the following month (Appendix B, I, and J Figures B1 B, I1 B, and J1 B). When the survival status of the honey bee colonies after the winter was compared to their positions on the PCA plot all of the colonies sampled in the first group were dead in February while the second group contained samples from two colonies that were dead and five colonies that were still alive in February (Figure 10 B). This separation by survival was not supported on the dendrogram where four colonies were 33.3% or more similar to a colony that had the opposite survival outcome after the winter (Figure 11 B).

When the June samples were compared by PCA two groups and five outliers were observed. However, none of the variables were able to explain the grouping of the bacterial profiles (Figure 10 C and Appendix B-J Figures B1 C, C1 C, D1 C, E1 C, F1 C, G1 C, H1 C, I1 C, and J1 C). The dendogram similarly to the PCA plot grouped multiple bee bread samples with the opposite winter survival outcome together, which verified that this factor did not explain the grouping seen (Figure 11 C).

One group and six outliers were seen when the bee bread samples in July were compared by PCA. The amount of bees, brood, mites, pollen, nectar, and honey did not reflect the grouping of the bee bread samples (Appendix B-G Figures B1 D, C1 D, D1 D, E1 D, F1 D, and G1 D). The brood pattern, queen status, and montly survival status also did not reflect the grouping of the bee bread samples (Appendix H-J Figures H1 D, I1D, and J1 D). When the survival status after winter was analyzed by PCA all samples in the group were from colonies that were dead after the winter except colony 18, which survived (Figure 10 D). All of the outliers were still alive after the winter except colony 4. The dendrogram also verified the similarity between the bacterial profiles of dead and surviving colonies (Figure 11 D). All bee bread samples showed similarity with another dead or surviving colony except colony 19, which was 43.5% similar to a surviving colony and colony 18, which survived was also similar to a dead colony by 23.5%.

The samples from August were positioned in two groups by PCA with two outliers. The queen status and montly survival status did not explain the variation because all of the colonies included in the analysis had a queen present and were all alive in August (Appendix I and J Figures I1 E and J1 E). The amount of bees, brood cells, mites, pollen, nectar, and honey did not explain the variation seen in August

(Appenedix B-G Figures B1 E, C1 E, D1 E, E1 E, F1 E, and G1 E). The survival status after the winter also did not reflect the bee bread bacterial profile positions (Figure 10 E). This was also verified by the dendrogram where two colonies were grouped with samples with opposite survival outcomes (Figure 11 E).

The PCA showed that the bacterial patterns of bee bread from the September sampling period were divided into two main groups with four outliers (Appendix B Figure B1 F). The amount of bees, brood cells, mites, pollen, nectar, honey did not reflect the grouping of the bee bread bacterial profiles (Appendix B- G Figures (B1 F, C1 F, D1 F, E1 F, and G1 F). The brood pattern, queen status, monthly survival status, and survival status after the winter also did not reflect the grouping of the bee bread bacterial profiles (Appendix H-J Figures H1 F, I1 F, and J1 F and Figure 10 F). The dendrogram showed that multiple bee bread samples from colonies that survived the winter were similar to colonies that died after the winter, verifying that the survival status after the winter did not reflect the grouping seen in September (Figure 11 F).

The PCA showed that the bacterial profiles of bee bread from the October sampling period were divided into three main groups with one outlier (Appendix B Figure B1 G). The amount of bees, brood cells, mites, pollen, nectar, and honey did not reflect the grouping of the bee bread bacterial profiles (Appendix B-G Figures B1 G, C1 G, D1 G, E1 G, F1 G, and G1 G). The survival status after the winter did not explain the grouping of the bee bread samples observed on the 2D PCA, however when a 3D PCA plot was used the bee bread samples from colonies that were dead were separated from colonies that were alive across the Z-axis (Figure 10 G and H). The dendogram more closely reflected the 2D PCA plot, which showed that four bee

bread samples from were grouped with colonies that had the opposite survival outcome (Figure 11 G).

3.5 FAME Analysis for the Identification of Bacteria

Five hundred forty-nine isolates were tested using FAME analysis (Figure 12 and Appendix K). One hundred fifty-eight of these organisms were identified because their Sim Index was above 0.600. Twenty-five *Enterobacteriaceae* species were also identified (Appendix K). Forty-nine additional organisms were identified at the genus level, or their exact species was not identified because there was less than a 0.100 Sim index difference from the next match. Sixty-seven organisms were identified from the *Enterobacteriaceae* family, but their Sim indexes had less than a 0.100 difference from the next match so the genus or species could not be identified. One hundred sixty-six organisms were not identified because their Sim index was below 0.600 and forty-three of the organisms were not identified because there was no match in the library.

Although only morphologically different organisms were isolated from the various media used, many species were repeatedly identified by FAME analysis. Of the 549 isolates 43 different species were identified (Table 3). Many organisms in the *Enterobacteriaceae* family were also identified; however most did not differ by a Sim index of at least 0.100 due to the family's similar fatty acid profiles. Though their close Sim index made them unable to be identified at the species level, the *Enterobacteriaceae* family was seen throughout the season (Table 3 and 4). The only genus identified throughout the entire season was *Bacillus* (Table 4). Within this genus, *Bacillus cereus* and *Bacillus megaterium* were the only species identified

throughout the season (Table 3). All of the other species were identified once or only in some of the months sampled during the season.

3.6 WalkAway® 40 for the Identification of *Enterobacteriaceae* Organisms

Ten of thirteen organisms were identified at the species level using the WalkAway® 40 (Table 5). *Pseudomonas fluorescence/putida* and *Enterobacter cloacae* were identified with 99.99% probability. *Enterobacter agglomerans* was identified with 97.43% probability. Eight of the organisms were identified as *E. agglomerans*, while one *P. fluorescence/putida* and one *E. cloacae* was identified. Two organisms could not be identified beyond the genus of *Enterobacter* because the percent probability was too low and one organism was not able to be identified at any level using the WalkAway® 40.

3.7 Antibiotic Susceptibility

Streptomycin, Oxytetracycline Hydrochloride, Tylosin Tartrate, Fumagilin-B, and Ampicillin were used to test for antibiotic resistance or susceptibility in thirteen organisms from the June sampling period. When 30 µg Oxytetracycline standard disks are used the zone of inhibition of resistant *Enterobacteriaceae* organisms is ≤ 14 mm and of sensitive organisms is ≥19 mm. Two organisms of the family *Enterobacteriaceae* had a zone of inhibition greater than 19 mm when exposed to the 25 µg disk so they would also be susceptible at the standard 30 µg concentration (Table 7). Three other organisms in the family *Enterobacteriaceae* had zones of inhibition of 18, 17, and 17 mm when organisms were exposed to 25 µg disks (Table 7). This suggests these organisms are susceptible or at most intermediately resistant to Oxytetracycline. Though the literature does not report *B. megaterium*'s minimum zone

of inhibition for resistance to Oxytetracycline, two different *B. megaterium* isolates responded differently when exposed to the Oxytetracyline disks. One isolate had no zone of inhibition when exposed to the 0.25 μg disk and had a 7.5 mm zone of inhibition in the presence of the 2.5 μg disk. The second isolate had an 8.5 mm zone of inhibition in the presence of the 0.25 μg disk and an 18.0 mm zone of inhibition in the presence of the 2.5 μg disk. The zones of inhibition for the controls E. *coli* and *S. aureus* were within the expected ranges.

At the standard concentration of 10 μg of Streptomycin a zone of inhibition of resistant *Enterobacteriaceae* organisms is ≤ 11 mm and of sensitive organisms is ≥15 mm. The Streptomycin disk potency of 2.5 μg resulted in a zones of inhibition that were greater than 11 for all *Enterobacteriaceae* organisms except for one (Table 8). At a disk potency of 25 μg all of the organsisms had a zone of inhibition greater than 15. This suggests that at the standard disk potency of 10 μg these organisms would be susceptible or at most intermittently resistant to Streptomycin. The two *B. megaterium* isolates again differed in their antibiotic sensitivity. One had a zone of inhibition when exposed to a 25 μg disk but none when exposed to a 2.5 μg or 0.25 μg disks. The other had zones of inhibition around all three disks. The controls *E. coli* and *S. aureus* had zones of inhibition within the expected ranges.

At the standard Ampicillin disk potency of 10 μg a zone of inhibition of resistant *Enterobacteriaceae* organisms is ≤ 11 mm and of sensitive organisms is ≥15 mm. The organisms of the family *Enterobacteriaceae* were resistant to Ampicillin, which was shown by no zone of inhibition at 2.5 μg or 25 μg (Table 9). 250 μg was the only concentration where a zone of inhibition was seen around these organisms. Similarly to the previous studies, one isolate identified as *B. megaterium* had a zone of

inhibition when exposed to a 2.5 µg Ampicillin disk while the other did not have a zone of inhibition until it was exposed to a 250 µg disk. The controls *E. coli* and *S. aureus* had zones of inhibition within the expected ranges.

The Tylosin Tartrate disk potencies of 2.5 and 25 µg were of levels representative of those found in the colony. At these concentrations the isolate identified as *Salmonella enterica* was the only member of the *Enterobacteriaceae* family to have a zone of inhibition around both the 0.25 and 2.5 disks (Table 10). All other isolates in this family had no zones of inhibition except in the presence of the 250 µg disk. The two isolates identified as *B. megaterium* again had zones of inhibition at different disk concentrations. One isolate had a zone of inhibition in the presence of the 0.25 µg disk while the other did not have any zones of inhibition at any concentration except 250 µg. The *E. coli* control had no zone of inhibition while *S. aureus* had zones of inhibition in the presence of 2.5, 25, and 250 µg disks.

Fumagillin-B also does not have a reported standard potency, however the manufacturer's recommended concentration of 25 mg/L calculates to 0.025 µg/µl. Disks were prepared to contain 025, 0.25, 2.5, 25 and 250 µg of Fumagillin-B. No zone of inhibition was observed for any of the organisms in the presence of the .025 µg disk (Table 11). Only one organism, which was an unidentified isolate, had a zone of inhibition at a disk potency of 250 µg. The *E. coli* control and *S. aureus* control also had no zone of inhibition at any disk potency of Fumagillin-B.

Chapter 4

DISCUSSION

4.1 DGGE is Useful in Comparitive Community Analysis Though Limitations Occur

The DGGE ladder was used to normalize DGGE gels by having a consistent banding pattern at both ends and in the middle of each gel. These banding patterns were then used to more accurately record the positions of DGGE profiles across gels. Though the 16S rRNA gene from pure isolates was amplified and tested by DGGE, multiple bands were seen in each lane containing one isolate. The occurrence of multiple DGGE bands for a single isolate have also been seen in other studies due to multiple heterogeneous 16S rRNA (59-63). Neilson et al. also found that multiple bands seen as a dublets occur as PCR artifacts (60). In this study when doubets were exised, reamplified, and reran on DGGE individually both bands were again seen. However, when the doublets were excised and reran on DGGE individually without reamplification only one band was seen. The cause of this phenomenon, however, is not understood. Due to the formation of artificial bands by PCR or DGGE it is suggested that this technique is used for comparity community analysis such as to observe microbial community shifts due to seasonal changes, bioremediation applications, or environmental pertubations (60). Since DGGE is still suitable for seasonal studies developing this technique would still be useful. However, the use of genetic sequencing would also be useful to verify the number of species and the differences seen across honey bee colonies.

4.2 Honey Bee Colony Survival is Presumably Due to Multiple Variables

When PCA was applied to all the bee bread bacterial banding patterns throughout the summer season no groups were seen. Additionally, none of the variables tested reflected the bee bread banding pattern positions. However, when the bee bread bacterial banding patterns were analyzed by month grouping of the bee bread samples was apparent and the survival status after the winter was the only factor that reflected the grouping. Research suggests that microorganisms may have an important role in honey bee colony health due to their ability to inhibit the colonization of pathogens and due to their production of antimicrobial peptides (2, 4, 64, 65). This inhibition of pathogen colonization is particularly important in the winter when honey bee colonies provide a habitat for pathogens, parasites, and viruses to grow without the ability of beekeepers to monitor or prevent their growth. Deformed Wing Virus and *Varroa destructor,* a virus and parasite that can be found in the colony, were both associated with a reduced life span of wintering bees in a study by Dainat et al. (66). Though American Foulbrood is not associated with winter losses, bacteria such as *Lactobacillus* and *Bifidobacterium* from the lactic acid bacteria group have been found to inhibit the growth of *Paenibacillus larvae*, which causes the disease, by stimulating the innate immune response in honey bees (64, 67). Our finding along with previous research therefore suggests that that the bacterial flora is also important for survival during the winter.

Though grouping was seen based on the survival status after the winter, this grouping was not seen in every month. Grouping by survival status after the winter was seen in May and July and in October on the 3D PCA plot. However, it was not seen in October on the 2D plot. Additionally, though the dendrograms accounted for intensity and position rather than just position in its analysis, the dendrograms had an

output similar to the PCA results of survival status after the winter in almost every month (Figure 10 B-H and Figure 11 B-G). The dendrogram also better reflected the results of the 2D plot suggesting that the 2D plot more accurately reflected the similarity of the bee bread samples based on survival status after the winter (Figure 10 G and H and Figure 11 G). Also, since the highest and second highest amount of variation is in the first and second component in PCA, it is possible that the grouping seen on the 3D plot was by chance.

One reason this grouping by survival status after the winter was not seen across all months is that all the variables were only considered individually in this study. Research has found that the current honey bee decline may be caused by multiple factors within colonies including parasites, pathogens, management stressors, and environmental stressors, but one factor leading to decline has not been identified (68). Similarly to honey bee decline it is likely that honey bee colony survival is based on multiple factors as well. This suggests that combining all of the factors in one analysis may provide further insight on variables involved in honey bee colony health. Additionally, using one analysis may determine if any of the variables that did not reflect the bee bread positions individually do reflect their placement when other variables are considered in combination. Though it is understood that the microbial flora plays an important role in the health of the colony other factors could have affected the honey bees' survival such as non-bacterial pathogens. Since this study only tracked and characterized the bacterial flora of bee bread it is possible that viruses or fungi that are pathogenic towards honey bees could have been present in the colony such as Deformed Wing Virus, and *Nosema apis*, or *Nosema cerane* (13). Therefore, though a separation was not seen in surviving and dead colonies in all

months, other areas of potential research could include determining what viruses or fungi are present in the colony since these can also affect their survival. Commensal molds and yeast are also found in the honey bee microenvironment and play important roles in the colony such as also providing antimicrobial properties in the colony, preventing the growth of pathogenic fungi, fermenting food stores when the bacterial flora are compormised, and synthesizing vitamins (3, 69). These roles also suggests the importance of considering molds and yeast in future studies on honey bee colony survival.

Though a separation was seen in certain months by PCA, these results again suggest that if PCR-DGGE can be used to predict the survival of a colony other factors must be involved. Across all the months it was seen that the number or variety of bands did not directly affect the survival outcome (Figure 10 A-H). Though symbiotic bacteria in the colony are understood to have many benefits such as competing with the colonization of pathogens, colonies with a greater number of bands, representing a larger bacterial flora did not indicate the colony survived the winter. Colonies that had bands absent that appeared common across the season also did not always indicate the colony died after the winter. One reason for this could be because PCR-DGGE is a finger print technique. Though the diversity of the bacteria in a colony could be tracked, this method could not differentiate what bacteria were commensal and which were pathogenic. Therefore, though a colony had a great diversity of bacteria, it also could have contained bacteria that were pathogenic towards bees such as *Pseudomonas aeruginosa* and *Enterococcus faecalis*, which were isolated and identified in this study (29).

Common bands were seen through this research suggesting common bacteria exist in bee bread across the season. However since a fingerprint technique could not identify what bacteria these bands represented, it is unknown what role they play in bee bread or in the health of the colony. Martha Gilliam found that microbial succession takes place as corbicular pollen is converted to bee bread. During this time the yeast and bacteria present vary over time based on the pH, and osmotic conditions of the bee bread (4). In this study Gilliam found that only *B. subtilis* was found in pollen from the flower, which suggests that the honey bees add many species of *Bacillus* (4). With time the number of isolates and species represented was also seen to decrease (22). This could suggest that colonies that had a low number of bands or absent common bands could have been due to the age of the bee bread samples rather than a lack of diversity in the bacterial flora in a colony. Therefore, future studies with fresh bee bread samples could be used to determine if the age of the pollen affects the bacterial flora seen in DGGE.

Research on the age of pollen would be particularly important since many studies suggest that honey bees develop a bacterial flora that does not vary by location (38, 70, 71). However, differences in the microbial flora of food stores that are close in spatial location to each other were seen in this study and in a study by Anderson et al. (34). They reported that bee bread sampled from neighboring colonies at the same time differed in their community structure exclusive of abundance. Apart from determining if pollen age accounts for these differences seen in the bacterial flora of neighboring colonies, genetics has been found to lead to bacterial differences in honey bee colonies. Mattila et al. found that when genetically diverse honey bee colonies were compared to honey bee colonies with a low level of diversity more unique

bacterial species were associated with the genetically diverse honey bee colonies (65). The genetically diverse colonies also had a higher number of beneficial genera and a lower number of sequences affiliated with genera known to be harmful when compared to the colonies with a low level of genetic diversity. This suggests that the genetic diversity of the honey bee colony may affect the bacterial flora present in the colony. Additionally, water homeostasis has been found to have a strong influence on the microbial balance of the honey bee colonies where the collection of water or pollen and nectar can be dependent on water need or genetic propensities respectively (3, 72, 73). Since trophyllactic interactions are necessary for the management of water, pollen, and nectar it is possible that these interactions also play a role in the bacterial flora of individual colonies (3). These findings in the literature suggest that these factors may account for differences seen in the bacterial flora of individual colonies each month.

4.3 Bacteria Found in Bee Bread are Associated with Diverse Habitats

Forty-three organisms were identified by the culture dependent method of FAME analysis. The organisms identified spanned twenty-six different genera. Early studies by Martha Gilliam et. al. used culture dependent methods to grow and identify organisms found in bee bread. This work showed the *Bacillus* species are the predominant bacteria found in bee bread (22). Additionally, this work suggested a microbial succession occurs in pollen due to the inoculation of bacteria by honey bees, which causes the microbial flora of floral pollen to be replaced. This was seen by bacteria identified as Gram-positive cocci, coryneforms, and Gram-negative rods that decreased in their abundances as pollen was converted to bee bread. The *Bacillus* species were identified in low quantities in floral pollen, but increased in abundance

and variety as pollen was converted to bee bread. This increase in variety was seen by the identification of *B. subtilis* alone in floral pollen, and the identification of *B. circulans*, *B. licheniformis*, *B. megaterium*, *B. pumilis*, *B. subtilis* and atypical *B. subtilis* stains in corbicular pollen (22). Early studies also identified species found in the intestine of the honey bee such as gram-variable pleomorphic bacteria (the taxonomic status was unknown) and *Enterobacteriaceae* (22). These microbes were reported to vary with the age of the bee, season, and geographical location, although some species of microorganisms were found consistently (22, 74). Additionally, most of the organisms isolated from corbicular pollen and bee bread were reported to be associated with the guts of adult worker bees (22).

Current studies have relied primarily on metagenomic sequencing to identify bacteria associated with honey bees. One study used barcoded amplicon pyrosequencing to research active (RNA producing) bacterial communities with a high level of genetic diversity (65). This study suggested that bee bread and the honey bee gut contain 207 species mutually as well as a many other species that were only found in bee bread or the gut alone (65). Other studies have led to the understanding that a core bacterial flora exists in the gut, which is composed of 8-12 bacterial strains. Many of these bacteria have been termed Lactic Acid Bacteria, which have been reported to have many beneficial roles in the colony including fermenting food substances, producing antimicrobial substances and inhibiting the growth of *Paenibacillus larvae*, the pathogen which causes American Foulbrood Disease (64). The flora of this group are composed of the genera *Lactobacillus* and *Bifidobacterium* (25). Later studies have shown that other organisms believed to be a part of the core

gut bacteria includes organisms of the family *Acetobacteraceae*, and of the genera *Pseudomonas*, *Bacillus*, and *Enterococcus* (3). Conflicting results indicated the role of Lactic Acid Bacteria in bee bread. Research by Oloffson et al. has shown that 11 of 12 strains of Lactic Acid Bacteria have also been identified in corbicular pollen and bee bread. It was proposed that these organisms aid in fermentation of these products and that their abundance decreases with time as bee bread gets more acidic (23). More recently, Anderson et al. found that although bee bread had the greatest diversity of bacteria compared to floral nectar, segments of the honey bee alimentary tract, honey, and pollen, its composition was primarily non-core gut bacteria. This study revealed bacteria found in bee bread most abundantly includes *Lactobacillus kunkeei*, and also species from the genera Firm 5 (most closely related to *Lactobacillus* spp.), *Enterococcus*, *Staphylococcus*, *Bacillus*, *Weissela*, and *Fructobacillus* (34).

Although these studies have revealed much information about bacteria in bee bread, this research has focused on the identification of bacteria throughout the summer season, which can provide information about transient or core bacteria found in bee bread. A total of twenty-six genera were represented by the forty-three species identified in bee bread. Of these genera, *Acetobacter, Bacillus, Citrobacter, Enterobacter, Enterococcus, Escherichia, Ewingella, Proteus, Pseudomonas, Rhodococcus,* and *Yersinia* have been identified in previous studies. To our knowledge this is the first time the fifteen other genera have been identified from bee bread.

4.3.1 Ubiquitous Bacteria in the Environment

The *Arthrobacter* spp. are among the most frequently isolated aerobic genera found in this environment. They typically are seen as Gram-negative rods in younger cultures and as Gram-positive cocci in older cultures (75). No literature to date has described this genus's role in honey bee colonies, however because *Arthrobacter* is ubiquitous, having been found in a variety of environments including common soils, arctic ice, and radioactive environments it may also be expected to be found in the colony (75). *Arthrobacter* spp. also survive long periods of stressful conditions including starvation, temperature shifts, and ionizing radiation. Since this genus was not found throughout the entire season it could be transient in honey bee colonies.

Bacillus was the most common genus isolated during the study and was the only one observed throughout the entire season. It is found ubiquitous in the environment and are Gram-positive or Gram-variable spore-forming rods (76). The G+C content of DNA of species within the genus can vary from 32-69% (76). Previous studies have identified it as a core organism in the honey bee gut and this study has determined it to be a core organism found in bee bread. Since *Bacillus* spp. are spore-formers they are resistant to adverse environmental conditions so it is not surprising to find them associated with colonies. *Bacillus* spp. produce antibiotics, terminally methyl-branched fatty acids, and many enzymes, which may be beneficial in bee bread, however their exact role in the conversion of pollen to bee bread is not yet understood. These microbial activities of *Bacillus* spp. may also have a significant role in bee bread throughout the season. The species of *Bacillus* identified in this study include *B. cereus*, *B. marisflavi*, *B. megaterium*, *B. mycoides*, *B. pumilus*, *B. thuringiensis*, and *B. subtilis*. In a study done by Martha Gilliam, *B. subtilis* was the

only species of *Bacillus* found in floral pollen, so it was suggested that honey bees add many species of *Bacillus* to bee bread (4).

Brevibacillus spp. include Gram-positive and Gram-variable endospore forming, aerobic and facultative anaerobic rod shaped bacteria (77). They were reclassified from the *Bacillus brevis* group in 1996 (77). Species in this genus are found in diverse environments including rocks, dust, aquatic environments, and guts of insects and other animals. *B. reuszeri* is a strictly aerobic, catalase positive, and oxidase negative organism with a GC content that ranges from 46.4-46.7% (77). *Brevundimonas* spp. belong to the class *Alphaproteobacteria* as a member of the family *Caulobacteraceae* (78). *B. vesicularis* was the only organism in this species identified in this study. It is a non-fermenting Gram-negative rod-shaped bacteria that is aerobic and motile (79). *B. vesicularis* is ubiquitous in the environment and has been isolated from water, aqueous solutions, and from clinical specimens from humans and animals, but it is rarely implicated in human infections (79). Its G+C DNA content ranges from 65-66%.

Citrobacter spp. are Gram-negative, motile bacteria (80). They are commonly found in water, soil, food, and intestinal tracts of humans and animals (80). Most infections caused by *Citrobacter* spp. are nosocomial, but they can also be community acquired (80). *C. freudii* was the only species in this genus identified and is often the cause of opportunistic infections (81). *Enterobacter* spp. are found in the soil and also in other habitats in the natural environment including water, sewage, and vegetables and they have also been identified in the intestine of honey bees (82, 83). Since the widespread use of antibiotics, members of this genus have been found to cause nosocomial infections (82). Resistance to the class of antibiotics, Cephalosporins has

also been seen in hospital settings likely because of increased β-lactamase production, which prevents the beta-lactam antibiotics from binding to bacterial penicillin-binding proteins (PBPs) (84, 85). *E. cloacae* was found in this study and is a Gram-negative bacterium that occurs as a commensal in water, soil, skin and hospital environments, however it has also been found in patient samples since the use of antibiotics (82). *E. hormaechei* is a Gram-negative rod that is often isolated from clinical sources. It has been shown to cause nosocomial infections and is associated with bloodstream infections (86). Since *Citrobacter* spp. and *Enterobacter* spp. are also found ubiquitously in the environment they can also be expected to be found in bee bread.

Enterococcus spp. are Gram-positive, catalase negative, non-spore-forming facultative anaerobic bacteria (87). They are found in the environment, in the alimentary tract of humans, and in animals (87). Species in this genus are able to live in extreme temperatures, pH concentrations, and NaCl concentrations, which allows them to survive in a range of niches (87). *Enterococcus* belong to the Lactic Acid Bacteria group, which are known to have a low G+C DNA content of less than 50% (87). *Enterococcus* spp. have been used in the food industry for its production of bacteriocins, but have recently become one of the most common nosocomial pathogens (87). *E. faecalis* was the only species in this genus identified and is an opportunistic pathogen commonly found in the gastrointestinal tract of humans, in the environment, and on animals. Therefore its presence in bee bread can be expected as well.

In this study *Pantoea agglomerans* and *P. ananatis* were isolated and identified. *Pantoea* spp. are often isolated from soil, but are also commonly isolated from other ecological niches including plants, water, humans, and animals (88). This

genus is often associated with plants as epiphytes or pathogens and can also cause disease in humans (88). *P. ananatis* causes disease symptoms in agricultural crops and forest tree species worldwide and is also able to infect humans (89). *P. agglomerans* is a Gram-negative plant pathogen and an opportunistic human pathogen that can occur sporadically or in outbreaks, however no literature currently indicates if *P. agglomerans* is pathogenic to honey bees (90).

P. vulgaris was the only organism in the *Proteus* genus isolated in this study. Organisms in the *Proteus* genus are motile, Gram-negative rods (91). *P. vulgaris* is widely distributed in the environment and has been found in the intestinal tract of mammals, birds, and reptiles (91). It is also found in the human gut and is a urinary tract pathogen (91). *Proteus* has previously been identified in the intestine of honey bees (83)

Pseudomonas is a genus found ubiquitously in the environment and is isolated from a variety of niches including plants, soil, water, and animals (92). Members of this genus are non-sporulating, aerobic Gram-negative rods (92). Many *Pseudomonas* species are pathogenic to plants and some strains are also pathogenic to animals (92). *P. aeruginosa*, which was identified in this study is an opportunistic pathogen (93). It can be isolated from environmental and hospital settings and can develop resistance to multiple classes of antibacterial agents during the course of therapy (93). *Pseudomonas aeruginosa*, is a honey bee pathogen and causes septicemia in adult honey bees (30). *P. florescens* was also identified in this study and is important for plant growth promotion and disease management by providing biological control of fire blight (94). It can be found in soil and water and is commonly associated with spoilage of foods. It can also be isolated from clinical specimens. *P. florescens* has a

low level of virulence, but some outbreaks of bacteremia in humans caused by *P. fluorescens* has been documented (95). *P. Putida* has also been isolated from soil and water, which are both environments where this organism can be transmitted to honey bees from.

Rhodoccocus are aerobic, Gram-positive, non-motile, mycolate- containing nocardioform actinomyceste (96). Organisms in this genus have been isolated from many sources including soil, rocks, groundwater, animal dung, healthy and diseased animals and plants and the guts of insects (96). They can also cause human, plant and animal diseases. *Rhodococcus* have become useful in environmental and industrial biotechnology because of its ability to transform and degrade chemicals (96). *R. equi* was identified in this study and is a pathogen of foal, which can lead to respiratory infections (96). *R. erythropolis* was also identified and is only known to cause disease in immunosupressed patients (96). This genus has previously been identified in bee bread (34).

Serratia has been isolated from many environments including soil, water, plants, humans, animals, and hospitalized human patients (97). Organisms in this genus are Gram-negative facultatively anaerobic rods (98). *Serratia* has also been associated with insects; however, *S. plymuthica*, which was identified in this study, is proposed to be primarily associated with water and was not found on insects (97, 99). *S. plymuthica* is also able to produce antimicrobial compounds and is used for biological control of fungal and bacterial plant pathogens (100).

S. parapaucimobilis and *S. sanguinis* were two organisms identified from the genus *Sphingomonas*. This genus has been isolated from many habitats including soil, hospital water supplies and equipment, blood, wounds, river water, drinking water,

and distilled water, deep surface sediments, corroding copper pipes, and the rhizosphere and surface of plants (101). Since *Sphingomonas* is also found ubiquitously in the environment it can also be expected to be found in bee bread. Most organisms in this family are Gram-negative aerobic heterotrophic organisms with a DNA G+C content ranging from 61-67% (101). *Sphingomomas* is often associated with plants, in which some strains exist as pathogens and others as antagonists against pathogens. *S. parapaucimobilis* shows antagonism against the phytopathogenic fungus *Verticillium dahliae*, which affects many commercially important plant species. *S. sanguinis* is also a member of the *Sphingomonas* genus and is often isolated from blood (78).

4.3.2 Bacteria Associated with Fruits, Vegetables, and Plants

Acetobacter is a genus of Gram-negative, obligate aerobic bacteria that are known as acetic acid bacteria due to their oxidation of ethanol that leads to the accumulation of acetic acid (102). Since their carbon sources include ethanol, glucose, and glycerol they are found occur in sugary, acidic, and alcoholic habitats. This suggests Acetobacter is found on a plant pollinated by honeybees. *Acetobacter* have a DNA G+C content of 52-61% across the species (102). *A. pasteurians* was one species from this genus identified during the study and is used in the industrial production of vinegar (102). It is isolated from wines and can also be found on ripe and injured grapes (102). *Pectobacterium carotovorum* was the only species of the *Pectobacterium* genus isolated during this study. It is a Gram-negative phytopathogen that causes soft root disease, wilt, and backleg in crops by secreting plant cell wall degrading enzymes (103, 104). It is rod shaped and has a G+C DNA content of 52.18% (104, 105). Though *P. carotovorum* has not been reported as a pathogen of

honey bees, since the habitat of this organism is plants it is likely that *P. carotovorum* could be transferred to honey bees when they visit plants for their pollen and nectar. This transfer of pathogenic organisms found on plants such as *Microbacterium*, *Pantoea, Pectobacterium*, and *Psedomonas*, which were identified in this study, could also lead to the movement of plant pathogens to other plants or other environments. Honey bees have been studied for use as vectors of microbiological control agents. These studies included the use of the *Glicoladium roseum*, which is antagonistic to the pathogenic fungus *Botrytis cinerea* in raspberry flowers, *Erwinia amylovora* and *Pseudomonas fluorescens*, which are anatonistic to fire blight of apples and pears, and *Bacillus thuringiensis*, which is antagonistic to the banded sunflower moth (94, 106, 107). Studies have also linked arthropod vectors as a mechanism of transmission of plant and animal viruses between hosts (108-110). This suggests that honey bees can also serve as vectors of plant and animal pathogens found in the colony.

Paenibacillus polymyxa was the only isolate of the *Paenibacillus* genus identified. It is a plant growth-promoting rhizobacteria with a broad host plant range, therefore P. *polymyxa* may have been transferred to bee bread during pollination of plants (111). It is Gram-positive, endospore-forming, and produces antibiotics. *P. polymyomxa* antagonizes pathogens such as oomycetic pathogens and *Arabidopsis thaliana* (105, 111). *Paenibacillus larvae*, another member of the *Paenibacillus* genus, was not identified in this study, but has been found to cause American Foulbrood disease in honey bees (13). However, current literature suggests *Paenibacillus polymyxa* has the potential to be used as a biocontrol agent and does not indicate it may also be pathogenic like its family member (111).

4.3.3 Bacteria Associated with Humans and Animals

Kocuria kristinae was the only spec*ies of Kocuria* identified during this study. This genus includes members that are Gram-negative and are strictly aerobic, although *Kocuria kristinae* is one exception, which is facultatively anaerobic (112). *Kocuria* spp. are catalase-positive, coagulate-negative, non-haemolytic cocci (112). Their DNA G+C content ranges from 60.0-75.3% depending on the species (112). *K. kristinae* is frequently found on the skin of humans (113). *Micrococcus luteus* was the only species of *Micrococcus* identified during the study. *Micrococcus* spp. are nonmotile and nonspore forming (114). They are Gram-positive cocci and their DNA G+C content ranges from 65-75% (114, 115). Soil was originally assumed to be the primary sources of *Micrococcus*, but soil is now known to contain small isolated populations of *Micococcus* (114). Mammalian skin is now considered the primary habitat of *Micrococcus* and in one study *M. lute*us was most commonly found member of this genus on human skin (114). *Micrococc*i have also been isolated from the skin of animals including squirrels, rats, raccoons, opossums, horses, swine, cattle, dogs, and primates. *M. varians* was found to be the predominant *Micococcus* species found on nonhuman mammalian skin while *M. luteus* was rarely isolated (114). This suggests *M. luteus* and *K. kristinae* could have been transferred from the skin of bee keepers.

The genus *Salmonella* is found in the digestive track of animals (116). However, when it is found in other habitats in the environment it is thought to be due by fecal contamination (116). *Salmonella* spp. are also foodborne pathogens, which can cause salmonellosis in humans. *S. enterica* was the only member of this genus identified during the study. This species includes Gram-negative, facultative intracellular, anaerobic rods that are further divided into subspecies based on their serotype (117).

4.3.4 Bacteria Associated with Clinical Specimens

Cedecea is a genus in the *Enterobacteriaceae* family. *C. davisae* was the only member of this genus identified and is known as an emerging pathogen (118). It is Gram-negative and has been previously been isolated from the sputum, gallbladder, and hand wounds (119). *C. davisae* has been implied in causing catheter-related blood stream infection, bacteremic skin and soft tissue infection, and lung infection. However, it has no reported roles in honey bees.

Escherichia coli was the only member of the *Escherichia* genus identified in this study. *E. coli* is a Gram-negative facultative anaerobe commonly found in the terminal small intestine and large intestine of mammals (120). They are occasionally isolated in association with the intestinal tract of nonmammalian animals and insects and can also be isolated from the environment including food, water, soil, and dust (120, 121). However, its presence in the environment is usually considered to reflect fecal contamination rather than its ability to replicate freely outside of the intestine (120). Some strains of *E. coli* contains pathogenicity islands in their chromosomes, which allows it to become virulent, however strains without it can have no pathogenic potential (120, 121). The G+C DNA content of its core genes typically ranges from 50-52% (120). *Escherichia* is commonly identified from the honey bee intestine (83).

Ewingella is a genus in the *Enterobacteriaceae* family. *E. Americana* is the only member of this genus and was identified in this study (122). Its pathogenicity and niches are not completely understood; however, this species has been identified in clinical specimens including wound, sputum, urine, stool, and blood and has also been identified in the intestine of honey bees (83, 122). *Kluyvera* is also a genus in the *Enterobacteriaceae* family. The members of this genus are Gram-negative, motile rods (123). They are catalase positive and oxidase negative (123). *K. ascorbata*, which

was identified in this study, is typically isolated from clinical specimen (123). *K. intermedia* has been isolated from surface water, soil, and a variety of human samples (124). Neither species has previously been identified in bee bread to our knowledge and its mode of transmission in the colony environment is not yet understood.

Microbacterium spp. are Gram-negative rods that have been isolated from clinical specimens (125). Both *M. barkeri* and *M. chocolatum* were identified from this genus. *Microbacterium* have been reported to cause human, animal and plant disease, but they have also been isolated from the soil and used as biocontrol agents (126). *Microbacterium* belongs to the phylum *Actinobacteria*, whose members have a high G+C DNA content (126). It has also not been previously found in bee bread, however because *Microbacterium* is found in a range of habitats its isolation from bee bread can be expected.

4.4 Antibiotic Susceptibility

Oxytetracycline HCl, Streptomycin, Tylosin Tartrate, Fumagillin-B, and Ampicillin were used in this study to determine the antibiotic susceptibility of bacteria found in bee bread. Based on the zone of inhibition around a 30 µg disk of Oxytetracyline the members of the *Enterobacteriaceae* family isolated in this study would be considered susceptible or at most have intermediate resistance to Oxytetracycline. The organisms in the *Enterobacteriaceae* family were also all considered susceptible or at most intermediately resitant to Streptomycin at its standard concentrations of 10 µg, but were resistant at Ampicillin's standard disk potency of 10 µg. The mode of action of both of these antibiotics is effective against Gram-positive and Gram-negative bacteria; however most organisms tested were resistant to Ampcillin until a disk potency of 250 µg was reached. Except for

Salmonella enterica, the organisms in the *Enterobacteriaceae* family were also resistant to Tylosin Tartate until a disk potency of 250 µg was used. This resistance was expected however because the mode of action of Tylosin Tartrate is effective against Gram-positive organisms. Though *S. enterica* is Gram-negative and was susceptible to Tylosin Tartrate it has been found that some Gram-negative organisms are affected by Tylosin (48). Fumagillin-B was uneffective against all bacteria tested except for one unidentified organism, which had a zone of inhibition when a disk potency of 250 µg was used. This resistance was expected, however since Fumagillin-B is effective against microspordia.

Since it is known that species in the *Enterobacteriaceae* family are present in honey bees, it would be useful to determine the roles of these species since the overuse of antibiotics can lead to resistance. Earlier reports have suggested that microorganisms belonging to *Enterobacteriaceae* are found in nectars or on pollens and contaminate bees during foraging rather than being symbionts in the honey bee intestine; however, more recent research identified members of *Enterobacteriaceae* in the intestine of honey bees (74, 83). In the early study by Martha Gilliam et al. the herbicide 2,4-D, Oxytetracycline and Fumagillin-B were used to determine the effects of 2,4-D on *Enterobacteriaceae* growth. Though the antibiotics were initially able to eliminate members of the *Enterobacteriacea*e, the organisms appeared again in later months of the study. The resistance of these organisms was not determined. Since *Enterobacteriaceae* isolated from bee bread are susceptible to Oxytetracycline and Streptomycin, which are used on bees and crops pollinated by honey bees, it would be beneficial to determine what roles these organisms play in the colony.

4.5 Conclusions

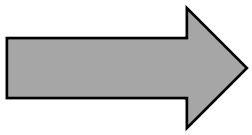
- The use of Gellan Gum growth media was able to cultivate the growth of a greater diversity and quantity of bacteria found in bee bread.
- The 43 species identified in this study showed that the bacterial flora of bee bread is diverse and consists of core organisms found in the honey bee gut and transient organisms presumably from the environment.
- *Enterobacteriaceae spp.* from bee bread were considered susceptible to Oxytetracycline and Streptomycin. Further research should be done on these species since their role in bee bread and in honey bees is not completely understood.
- The two bee bread pathogens and eleven plant, human, and animal pathogens isolated demonstrated that some pathogens can be commensal in different host. Some plant, human, and animal pathogens are reported to not be pathogenic to bees or were characterized from the bacterial flora of a colony that survived.
- The bacterial flora of bee bread can be tracked by PCR-DGGE and varies as the season progresses and can differ from neighboring honey bee colonies within the same sampling period.
- Principal component analysis can be used to determine the similarity of bacterial profiles found in bee bread.
- The similarity of the bacterial flora of bee bread is not reflective of the amount of pollen, nectar, or honey found in the honey bee colony.
- Though the number of bees, brood, and mites as well as the brood pattern and queen status affect the strength of the colony they did not reflect the similarity of the microflora of bee bread.

- The bacterial flora of bee bread may be one of multiple indicators of honey bee colony survival. Other factors are also likely involved and should be evaluated include the presence of viruses or pathogenic fungi, or the age of the bee bread sampled.

TABLES

Table 1: Honey bee colonies that were used during the study. The first table indicates all 32 honey bee colonies from which bee bread was sampled from May 2013-October 2013. The second column indicates the 15 honey bee colonies from which bee bread was used in the study for all DGGE and FAME analysis work.

Bee colonies sampled	
1B1	17B1
2A2	18B1
3B1	19B2
4B2	20A1
5A2	21B1
6B1	S1B
7A1	S3
8B1	S4
9A2	S6
10A2	S12
11A1	S15
12B2	S8
13A1	S18(1)
14A2	S18(2)
15B2	S19
16A2	S21



Bee colonies used in study
2A2
s3B1
4B2
7A1
9A2
11A1
12B2
s12B2
13A1
14A2
16A2
18B1
19B2
20A1
21B1

Table 2: Table of bee bread samples tested by PCR-DGGE. Green boxes indicate PCR products observed on 2% agarose gels were sufficient for DGGE. Dark grey cells indicate no PCR product was observed on 2% agarose gels. Light grey cells indicate the colony died before the end of the study and that bee bread was not present for further testing.

	May	June	July	Aug	Sept	Oct
2A2			Died			
s3B1					Died	
4B2						
7A1						
9A2						
11A1						
12B2						
s12B2						
13A1						
14A2						
16A2						
18B1						
19B2						Died
20A1						
21B1						

Table 3: Bacteria identified from bee bread of honey bee colonies from the University of Delaware research apiary by FAME analysis by month. * indicates the species could not be distinguished by FAME analysis because the Similarity Index (Sim Index) of the two species had less than a 0.100 difference. All other species had a Sim Index of at least 0.600 and a separation from the next match by at least 0.100, which is acceptable for identification. M, Je, Jy, A, S, and O represent May, June, July, August, September, and October respectively.

Species	M	Je	Jy	A	S	O
Acetobacter pasteruianus						
Arthrobacter nicotianae						
Bacillus atrophaeus						
Bacillus cereus						
Bacillus circulans						
Bacillus marisflavi						
Bacillus megaterium						
Bacillus megaterium or *Brevabacillus parabrevis**						
Bacillus mycoides						
Bacillus pumilus						
Bacillus sphaericus						
Bacillus subtilis						
Bacillus thuringiensis						
Brevibacillus reuszeri						
Brevundimonas vesicularis						
Cedecea davisae						
Citrobacter freudii						
Enterobacter hormaechei						
Enterococcus faecalis						
Escherichia coli						
Ewingella Americana						
Flavimonas oryzihabitans or *Chryseomonas luteola**						
Flavimonas oryzihabitans or *Pseudomonas aeruginosa**						

Kluyvera ascorbata						
Kluyvera intermedia						
Kocuria kristinae						
Microbacterium barkeri						
Microbacterium chocolatum						
Microbacterium lacticum or *Clavibacter michiganensis**						
Micrococcus luteus						
Paenibacillus polymyxa						
Pantoea agglomerans						
Pantoea ananatis						
Pectobacterium carotovorum						
Proteus vulgaris						
Pseudomonas aeruginosa						
Pseudomonas aeruginosa or *Flavimonas oryzihabitans**						
Pseudomonas putida						
Rhodococcus equi						
Rhodococcus erythropolis						
Salmonella enterica						
Serratia plymuthica						
Sphingomonas parapaucimobilis						
Sphingomonas sanguinis						
Staphylococcus hominis						
Staphylococcus xylosus						
Yersinia aldovae						
Yersinia bercovieri						
Genera						
Enterobacter genus						
Micrococcus genus						
Pseudomonas genus						
Sphingomonas genus						
Staphylococcus genus						
Family						

Enterobacteriaceae						
Other						
Not identified- sim index too low						
No match						

Table 4: Bacteria identified from bee bread from honey bee colonies sampled monthly at the University of Delaware research apiary by FAME analysis shown at the genus level. * indicates the genus could not be distinguished by FAME analysis because the Similarity Index (Sim Index) of two species had less than a 0.100 difference. All genera were identified at the species level and had a Sim Index of at least 0.600 and a separation from the next match by at least 0.100, which is acceptable for identification. M, Je, Jy, A, S, and O represent May, June, July, August, September, and October respectively.

Genera	M	Je	Jy	A	S	O
Acetobacter		■				■
Arthrobacter	■		■			■
Bacillus	■	■	■	■	■	■
Bacillus or *Brevabacillus**	■					
Brevibacillus	■				■	
Brevundimonas					■	
Cedecea				■	■	
Citrobacter					■	
Enterobacter		■				
Enterococcus				■		
Escherichia					■	
Ewingella				■		■
Flavimonas or *Chryseomonas**				■		
Flavimonas or *Pseudomonas**				■		
Kluyvera		■		■	■	
Kocuria				■		
Microbacterium	■			■	■	
Microbacterium or Clavibacter*						■
Micrococus	■	■		■		
Paenibacillus			■			■
Pantoea		■			■	
Pectobacterium					■	
Proteus			■			

Pseudomonas						
Pseudomonas or *Flavimonas**						
Rhodococcus						
Salmonella						
Serratia						
Sphingomonas						
Staphylococcus						
Yersinia						
Family						
Enterobacteriaceae						
Other						
Not identified- sim index too low						
No match						

Table 5: Organisms isolated in June from the South Campus Research Apiary and identified using the WalkAway® 40 System. The number of organisms identified indicates how many isolates were identified as a given species. * indicates there was a low probability of identification so the genus and species could not be determined for the isolate. ** indicates there were no matches in the library for the isolate.

Organism Identification	**Percent Probability**	**Number of Organisms Identified**
Pseudomonas fluorescence/putida	99.99	1
Enterobacter agglomerans	97.43	8
Enterobacter cloacae	99.99	1
Enterobacer Species	Low Probablility ID*	2
No ID	n/a	1

Table 6: Organisms identified by FAME analysis and the WalkAway® 40 System organized by habitat. Genera with species identified that can are pathogenic or opportunistic pathogens in its respective habitat are shown in red. Genera that were isolated from bee bread for the first time in this study or genera with species that have been identified for the first time in bee bread are made bold. Green boxes indicate at least one genus from a given habitat was present and dark grey indicates no genera were present from a given habitat.

Habitat	M	Je	Jy	A	S	O	Genera
Ubiquitous in environment (ex. soil, air, water)							Arthrobacter, Bacillus, **Brevibacillus**, **Brevundimonas**, Citrobacter, Enterobacter, Enterococcus,**Kluyvera**, **Microbacterium**, **Pantoea**, Proteus, Pseudomonas, **Serratia**, **Sphingomonas**, Rhodococcus
Fruits, vegetables, and flowers							Acetobacter, Enterobacter, **Microbacterium**, **Paenibacillus**, **Pantoea**, **Pectobacterium**, Pseudomonas, **Sphingomonas**
Humans and Animals							Citrobacter, Enterobacter, **Kocuria**, **Microbacterium**, **Micrococcus**, **Pantoea**, Pseduomonas, **Salmonella**
Clinical Specimens							**Cedecea**, Escherichia, Ewingella, **Kluyvera**, Proteus, **Salmonella**, **Serratia**

Table 7: The zone of inhibition of bacteria isolated from bee bread in Oxytetracycline antibiotic susceptibility testing. Lanes highlighted blue, green, white, and orange are the antibiotic susceptibility testing results of *Enterobacteriaceae*, *Bacillus megaterium*, unidentified organisms due to their Sim index being too low, and organisms with no match in the Sherlock® Microbial Identification System respectively. *E. coli* and *S. arueus* were used as controls and are highlighted yellow.

Disk Potency of Oxytetracycline HCl (µg/µl)	**0**	**0.025**	**0.25**	**2.5**	**25**	**250**
71E20-Enterobacteriaceae	0	0	0	9	18	22
72E20-Salmonella enterica	0	0	8	13	21	25
73E20-Enterobacteriaceae	0	0	0	10	17	20
74-1E20-Bacillus megaterium	0	0	8.5	18	24	30
75-1E20-No match	0	0	0	7.5	12	16
75-2E20-Sim index too low	0	0	7.5	14	21	26
75-3E20-Enterobacteriaceae	0	0	0	12	18	23
76-1E20-Bacillus megaterium	0	0	0	7.5	13	16
81-1E20-Enterobacteriaceae	0	0	0	14	20	23
81-2E20-Enterobacteriaceae	0	0	0	11	17	23
85E20-Sim index too low	0	0	7.5	18	22.5	27
E. coli	0	0	0	14	18	22
S. aureus	0	0	15	26	34	40

Table 8: The zone of inhibition of bacteria isolated from bee bread in Streptomycin antibiotic susceptibility testing. Lanes highlighted blue, green, white, and orange are the antibiotic susceptibility testing results of *Enterobacteriaceae*, *Bacillus megaterium*, unidentified organisms due to their Sim index being too low, and organisms with no match in the Sherlock® Microbial Identification System respectively. *E. coli* and *S. arueus* were used as controls and are highlighted yellow.

Disk Potency of Streptomycin (µg/µl)	0	0.025	0.25	2.5	25	250
71E20-Enterobacteriaceae	0	0	0	12	17	23
72E20-Salmonella enterica	0	0	0	12	18	23
73E20-Enterobacteriaceae	0	0	0	12	18	24
74-1E20-Bacillus megaterium	0	0	10	20	25	30
75-1E20-No match	0	0	0	0	14	22
75-2E20-Sim index too low	0	0	0	14	21	30
75-3E20-Enterobacteriaceae	0	0	0	11	18	25
76-1E20-Bacillus megaterium	0	0	0	0	13	21
81-1E20-Enterobacteriaceae	0	0	0	10	17	23
81-2E20-Enterobacteriaceae	0	0	0	11	17	24
85E20-Sim index too low	0	0	7.5	14	19	26
E. coli	0	0	0	10	15	19
S. aureus	0	0	0	8	15	21

Table 9: The zone of inhibition of bacteria isolated from bee bread in Ampicillin antibiotic susceptibility testing. Lanes highlighted blue, green, white, and orange are the antibiotic susceptibility testing results of *Enterobacteriaceae*, *Bacillus megaterium*, unidentified organisms due to their Sim index being too low, and organisms with no match in the Sherlock® Microbial Identification System respectively. *E. coli* and *S. arueus* were used as controls and are highlighted yellow.

Disk Potency of Ampicillin (µg/µl)	0	0.025	0.25	2.5	25	250
71E20-*Enterobacteriaceae*	0	0	0	0	0	9
72E20-*Salmonella enterica*	0	0	0	0	0	12
73E20-*Enterobacteriaceae*	0	0	0	0	0	16
74-1E20-*Bacillus megaterium*	0	0	0	17	26.5	32
75-1E20-No match	0	0	0	0	0	13
75-2E20-Sim index too low	0	0	0	0	0	12
75-3E20-*Enterobacteriaceae*	0	0	0	0	0	11
76-1E20-*Bacillus megaterium*	0	0	0	0	0	15
81-1E20-*Enterobacteriaceae*	0	0	0	0	0	10
81-2E20-Enterobacteriaceae	0	0	0	0	0	11
85E20-Sim index too low	0	0	0	16	21	25
E. coli	0	0	0	0	17	23
S. aureus	0	0	19	29	40	44

Table 10: The zone of inhibition of bacteria isolated from bee bread in Tylosin Tartrate antibiotic susceptibility testing. Lanes highlighted blue, green, white, and orange are the antibiotic susceptibility testing results of *Enterobacteriaceae*, *Bacillus megaterium*, unidentified organisms due to their Sim index being too low, and organisms with no match in the Sherlock® Microbial Identification System respectively. *E. coli* and *S. arueus* were used as controls and are highlighted yellow.

Disk Potency of Tylosin Tartrate (µg/µl)	0	0.025	0.25	2.5	25	250
71E20-Enterobacteriaceae	0	0	0	0	0	0
72E20-Salmonella enterica	0	0	10	17	26	29
73E20-Enterobacteriaceae	0	0	0	0	0	0
74-1E20-Bacillus megaterium	0	0	7.5	15	22	28.5
75-1E20-No match	0	0	0	0	0	9.5
75-2E20-Sim index too low	0	0	7.5	11.5	23	30
75-3E20-Enterobacteriaceae	0	0	0	0	0	0
76-1E20-Bacillus megaterium	0	0	0	0	0	10
81-1E20-Enterobacteriaceae	0	0	0	0	0	9
81-2E20-Enterobacteriaceae	0	0	0	0	0	15
85E20-Sim index too low	0	0	0	0	10	15
E. coli	0	0	0	0	0	0
S. aureus	0	0	0	11	21	30

Table 11: The zone of inhibition of bacteria isolated from bee bread in Fumagillin-B antibiotic susceptibility testing. Lanes highlighted blue, green, white, and orange are the antibiotic susceptibility testing results of *Enterobacteriaceae*, *Bacillus megaterium*, unidentified organisms due to their Sim index being too low, and organisms with no match in the Sherlock® Microbial Identification System respectively. *E. coli* and *S. arueus* were used as controls and are highlighted yellow. 100% Ethanol (EtOH) was used as a vehicle control.

Disk Potency of Fumagillin-B (µg/µl)	0	0.025	0.25	2.5	25	250	EtOH
71E20-Enterobacteriaceae	0	0	0	0	0	0	0
72E20-Salmonella enterica	0	0	0	0	0	0	0
73E20-Enterobacteriaceae	0	0	0	0	0	0	0
74-1E20-Bacillus megaterium	0	0	0	0	0	0	0
75-1E20-No match	0	0	0	0	0	0	0
75-2E20-Sim index too low	0	0	0	0	0	12	0
75-3E20-Enterobacteriaceae	0	0	0	0	0	0	0
76-1E20-Bacillus megaterium	0	0	0	0	0	0	0
81-1E20-Enterobacteriaceae	0	0	0	0	0	0	0
81-2E20-Enterobacteriaceae	0	0	0	0	0	0	0
85E20-Sim index too low	0	0	0	0	0	0	0
E. coli	0	0	0	0	0	0	0
S. aureus	0	0	0	0	0	0	0

FIGURES

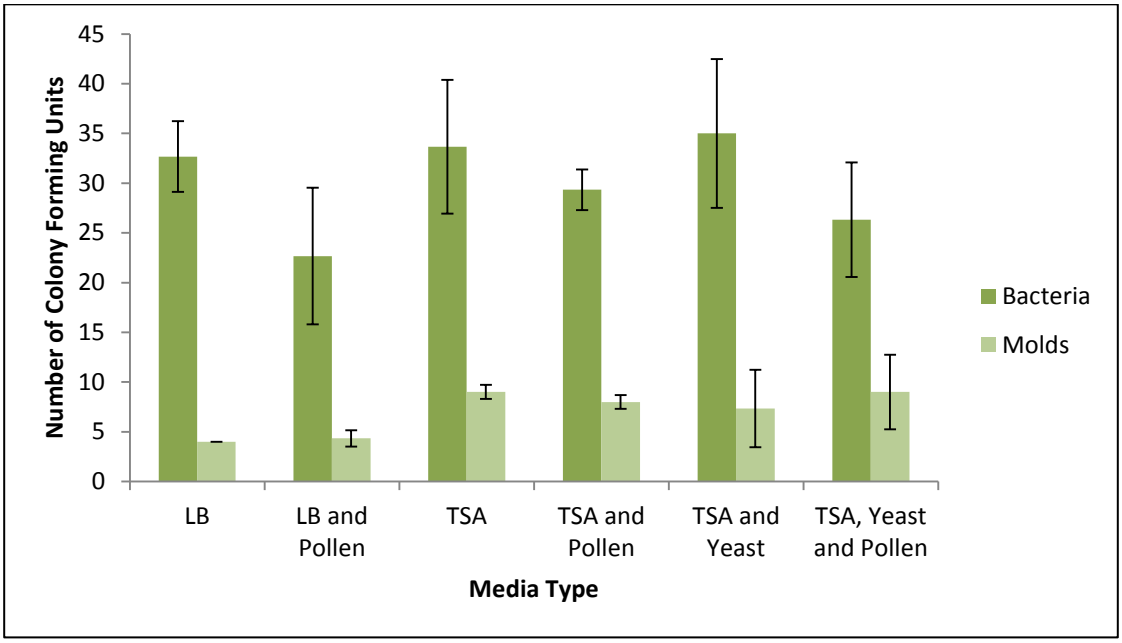


Figure 1: Bacterial and fungal colony forming units after 48 hours of incubation. Colonies were counted after 48 hours of incubation on Luria-Bertani Agar (LB), LB with pollen, Tryptic Soy Agar (TSA), TSA with pollen, TSA with yeast, or TSA with yeast and pollen. Bars represent SEM.

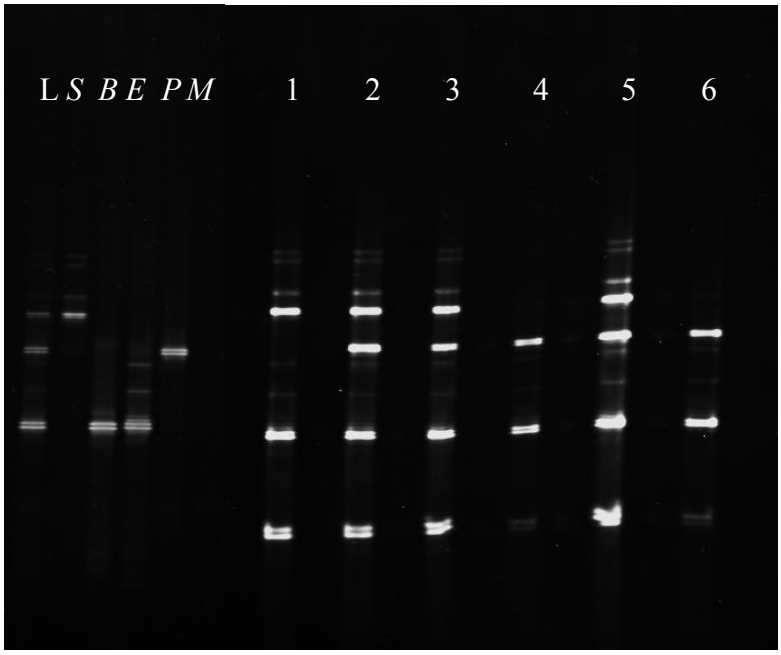


Figure 2: A.) DGGE Gel of DGGE ladder (L) and organisms in DGGE ladder: *Staphylococcus aureus (S), Bacillus subtilis (B), Escherichia coli (E), Pseudomonas aeruginosa (P), and Micrococcus luteus (M)* respectively. B.) DGGE gel of DGGE ladder comparing DNA of ladders after ethanol precipitation to DNA without ethanol precipitation. Lanes 1, 2, 3, 4, 5, and 6 contained 6, 8, 7, and 7, 10, and 10 µl of each organism. Lanes 4 and 6 did not have an ethanol precipitation done after DNA extraction.

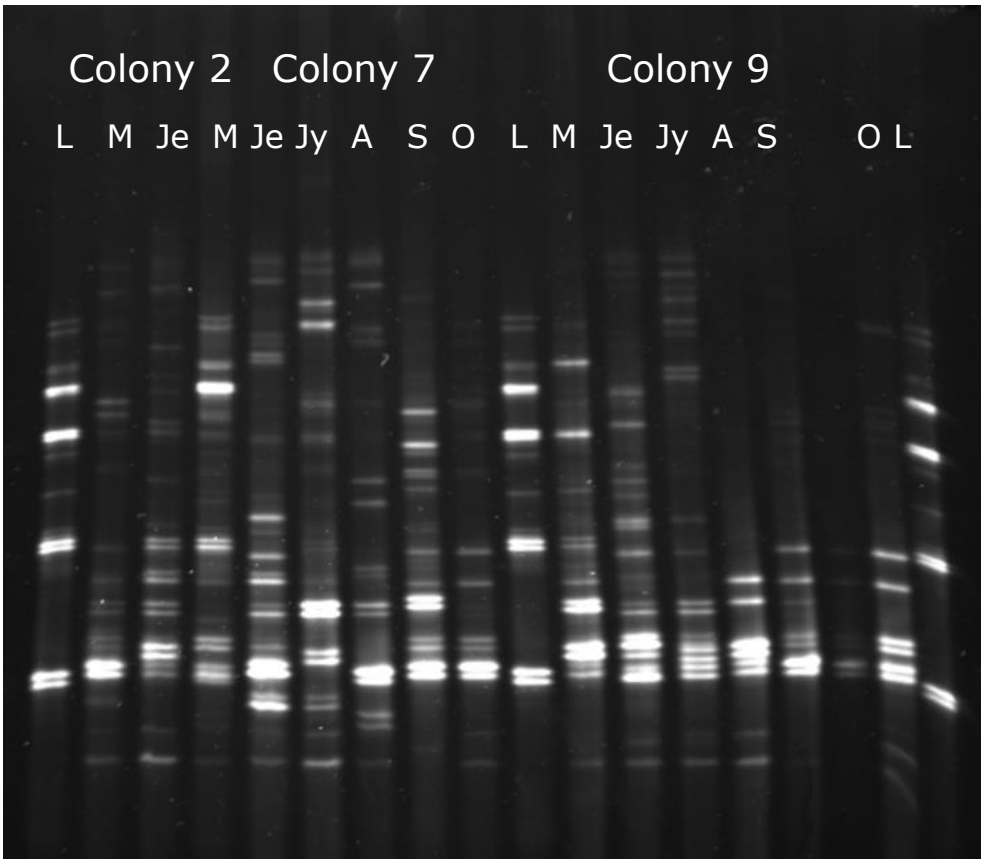


Figure 3: DGGE of colonies 2, 7, and 9 from May to October 2013. L indicates the DGGE ladder, M - the bee bread sample was from May, Je - the bee bread sample was from June, Jy - the bee bread sample was from July, A - the bee bread sample was from August, S - the bee bread sample was from September, and O - the bee bread sample was from October.

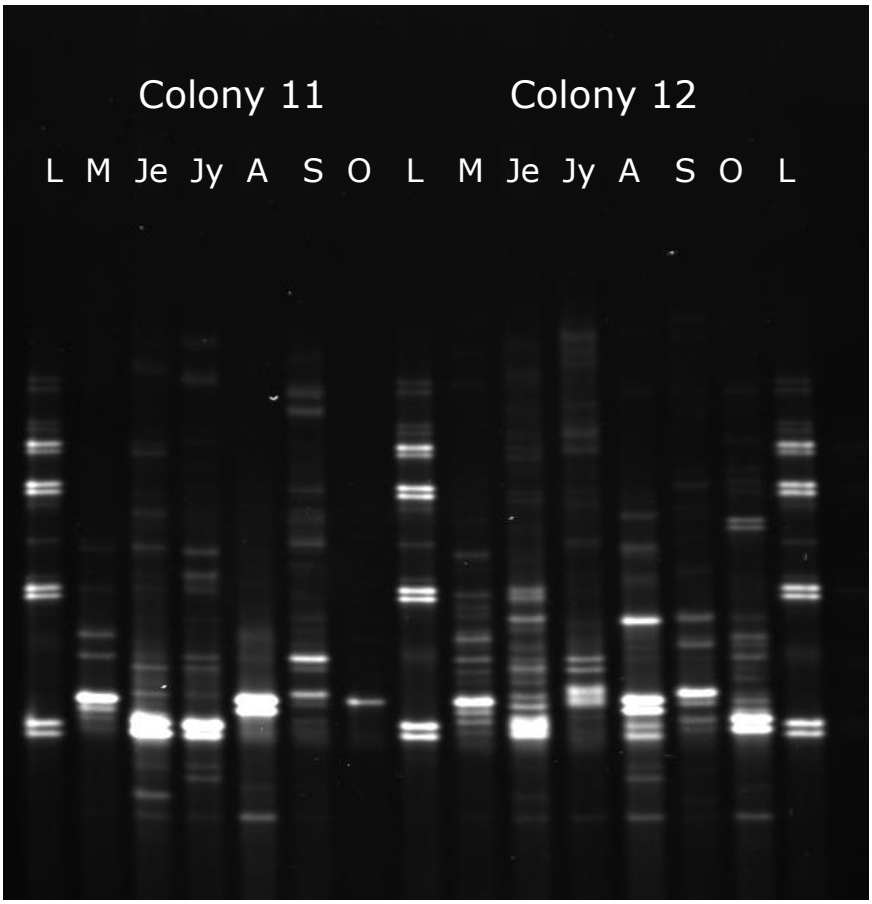


Figure 4: DGGE of bee bread from colonies 11 and 12 from May to October 2013. L indicates the DGGE ladder, M - the bee bread sample was from May, Je - the bee bread sample was from June, Jy - the bee bread sample was from July, A - the bee bread sample was from August, S - the bee bread sample was from September, and O - the bee bread sample was from October.

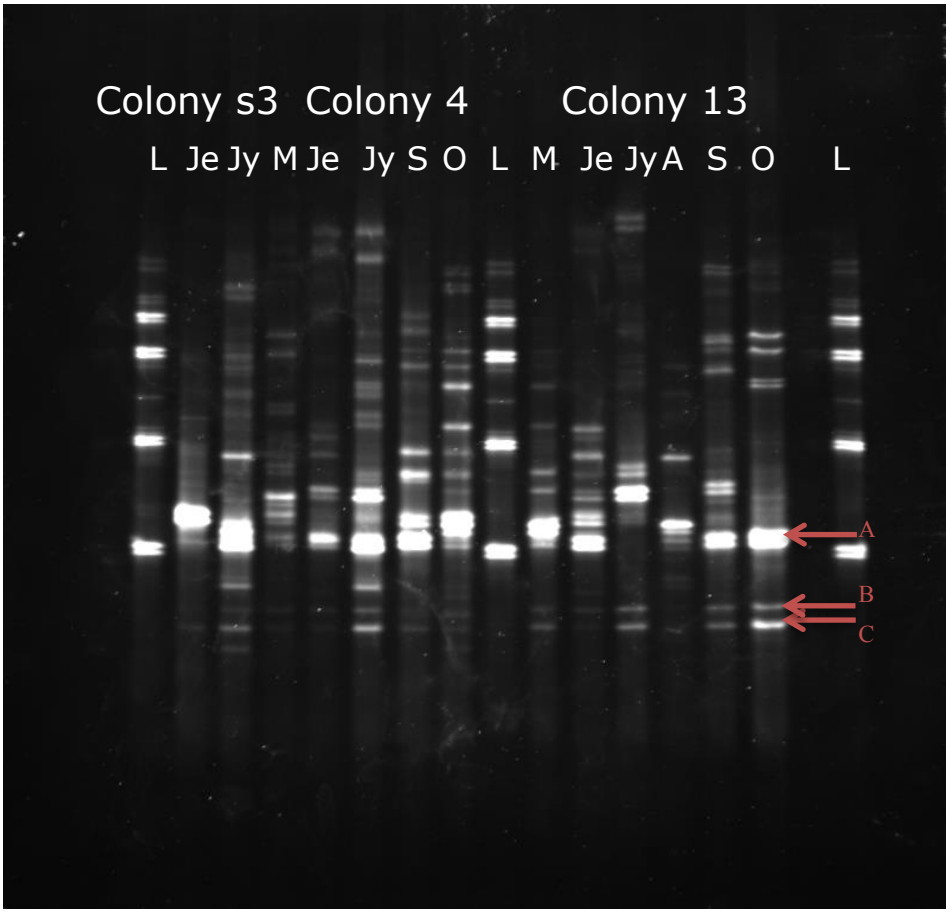


Figure 5: DGGE of bee bread from colonies s3, 4, and 13 from May to October 2013. Arrow A indicates the band within the same DNA G+C content as the DGGE ladder organism *M. luteus* that was common across most bee bread samples. Arrow B and C - the two common bands with the lowest DNA G+C content seen across most bee bread samples. L indicates the DGGE ladder, M - the bee bread sample was from May, Je - the bee bread sample was from June, Jy - the bee bread sample was from July, A - the bee bread sample was from August, S - the bee bread sample was from September, and O - the bee bread sample was from October.

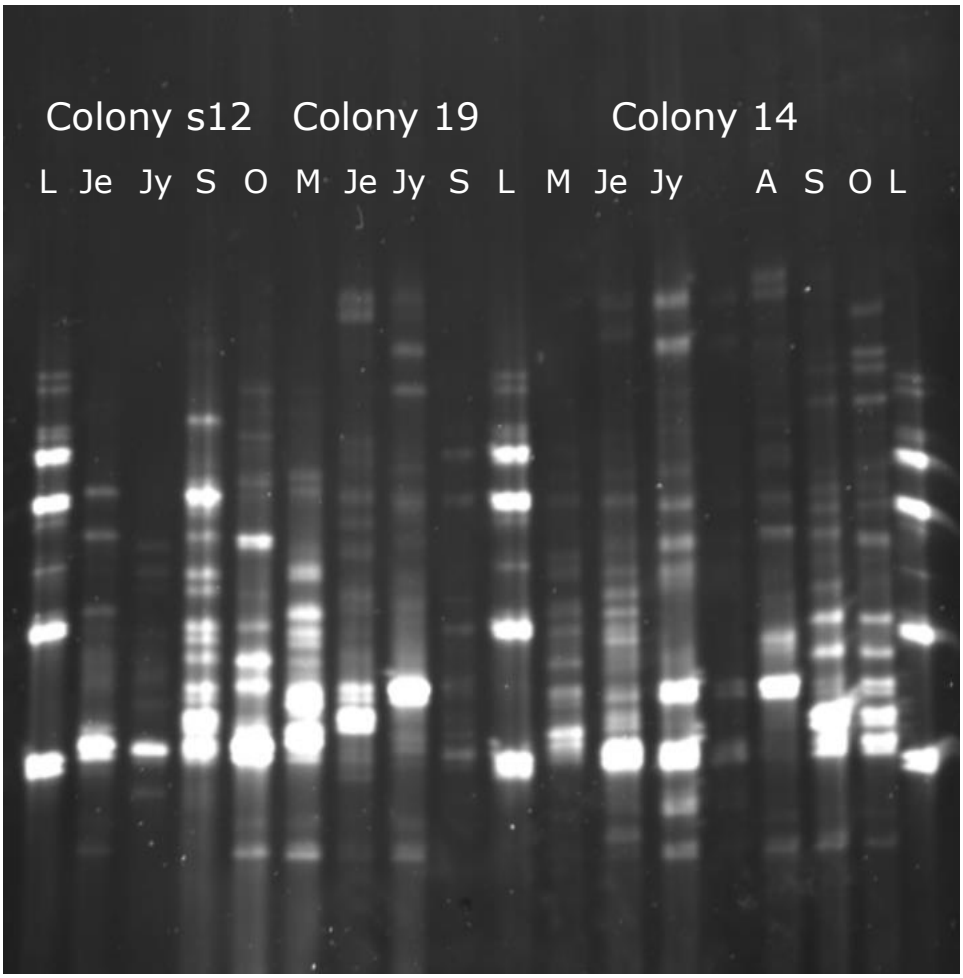


Figure 6: DGGE of bee bread from colonies s12, 19, and 14 from May to October 2013. L indicates the DGGE ladder, M - the bee bread sample was from May, Je - the bee bread sample was from June, Jy - the bee bread sample was from July, A - the bee bread sample was from August, S - the bee bread sample was from September, and O - the bee bread sample was from October.

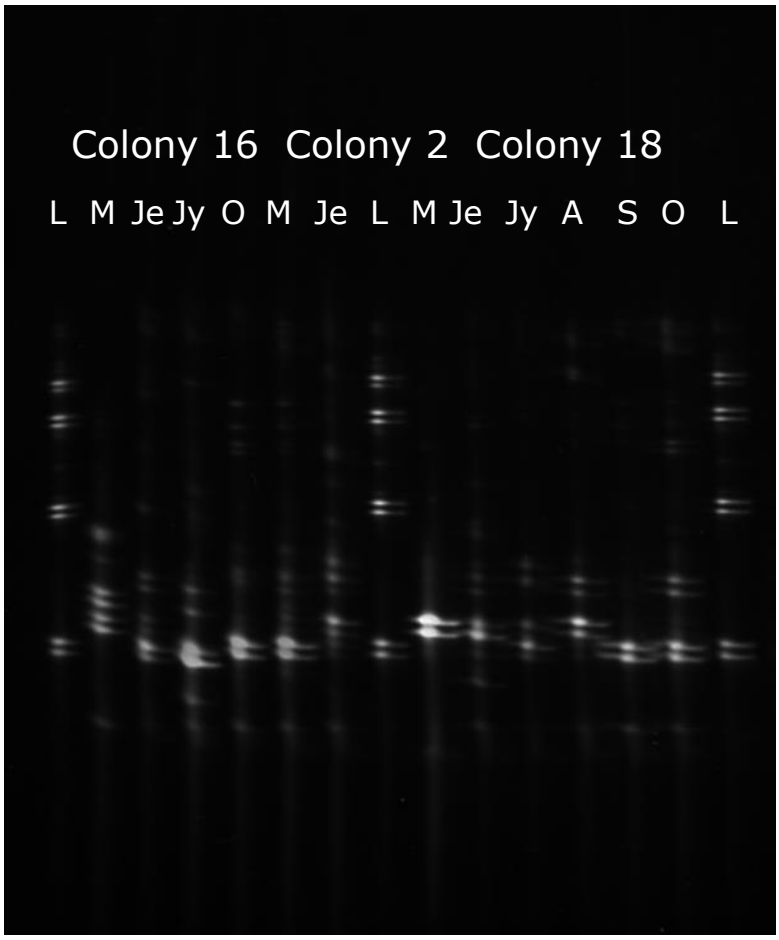


Figure 7: DGGE of bee bread from colonies 16, 18 and 2 from May to October 2013. L indicates the DGGE ladder, M - the bee bread sample was from May, Je - the bee bread sample was from June, Jy - the bee bread sample was from July, A - the bee bread sample was from August, S - the bee bread sample was from September, and O - the bee bread sample was from October.

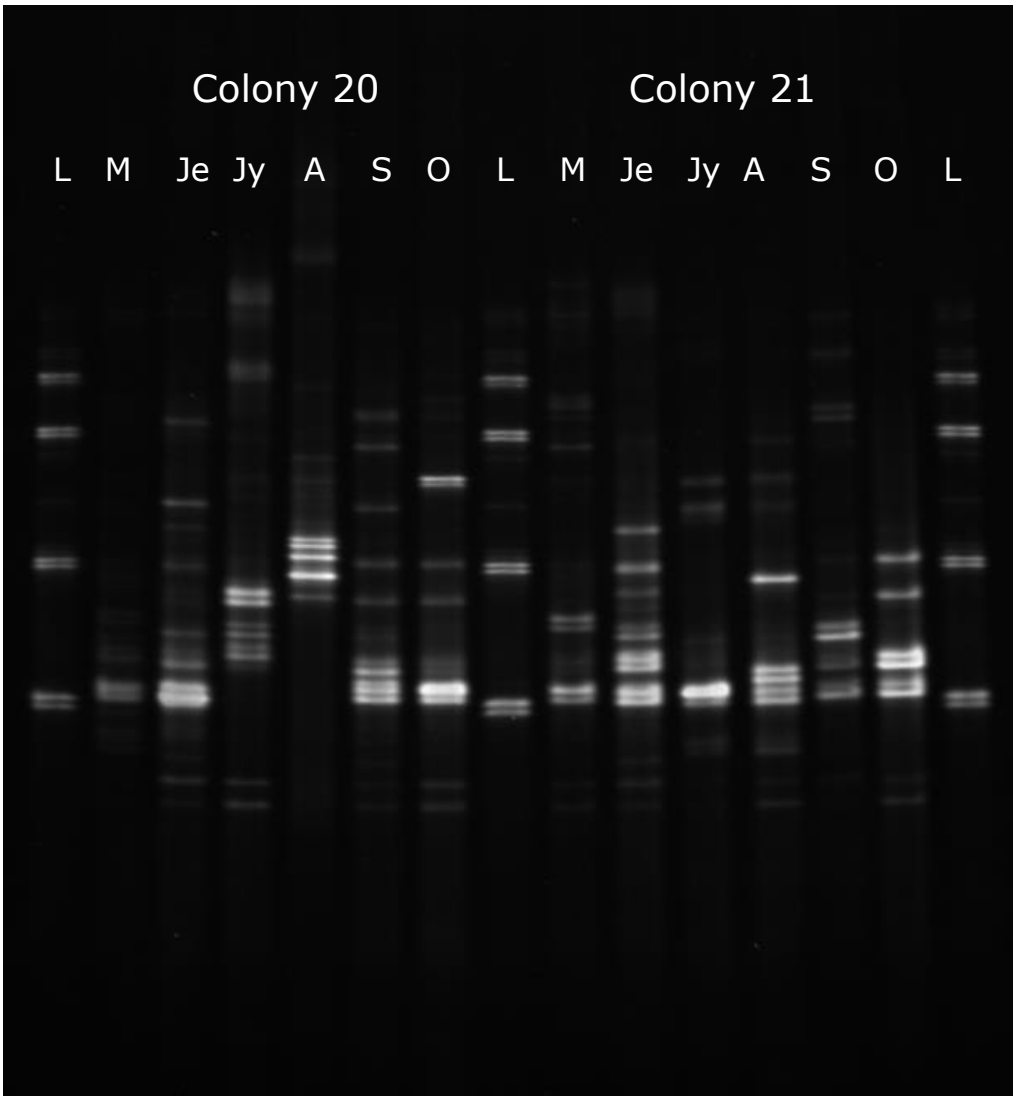


Figure 8: DGGE of bee bread from colonies 20 and 21 from May to October 2013. L indicates the DGGE ladder, M - the bee bread sample was from May, Je - the bee bread sample was from June, Jy - the bee bread sample was from July, A - the bee bread sample was from August, S - the bee bread sample was from September, and O - the bee bread sample was from October.

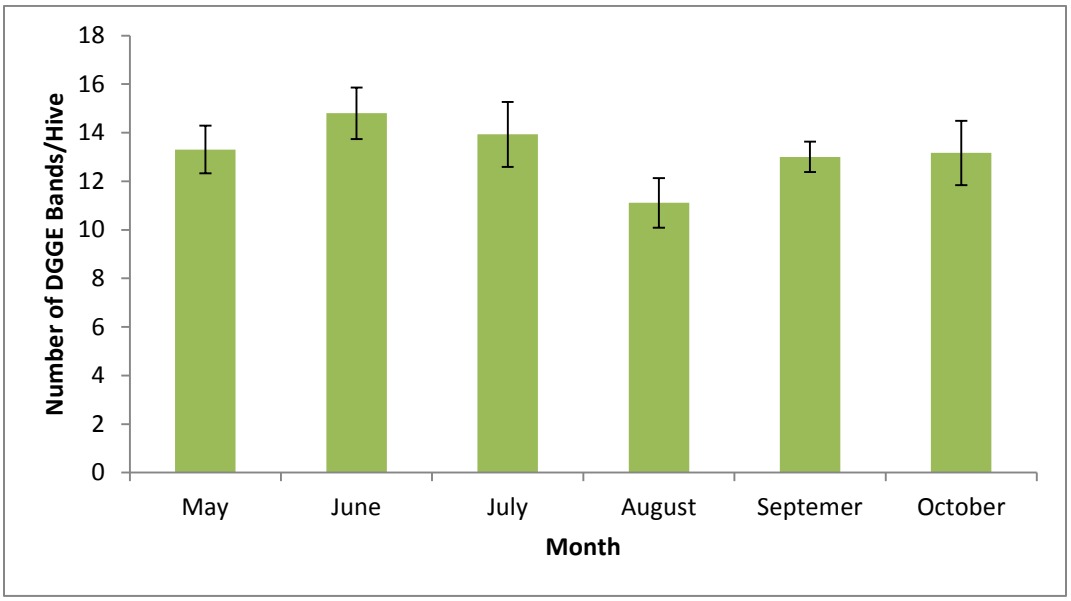
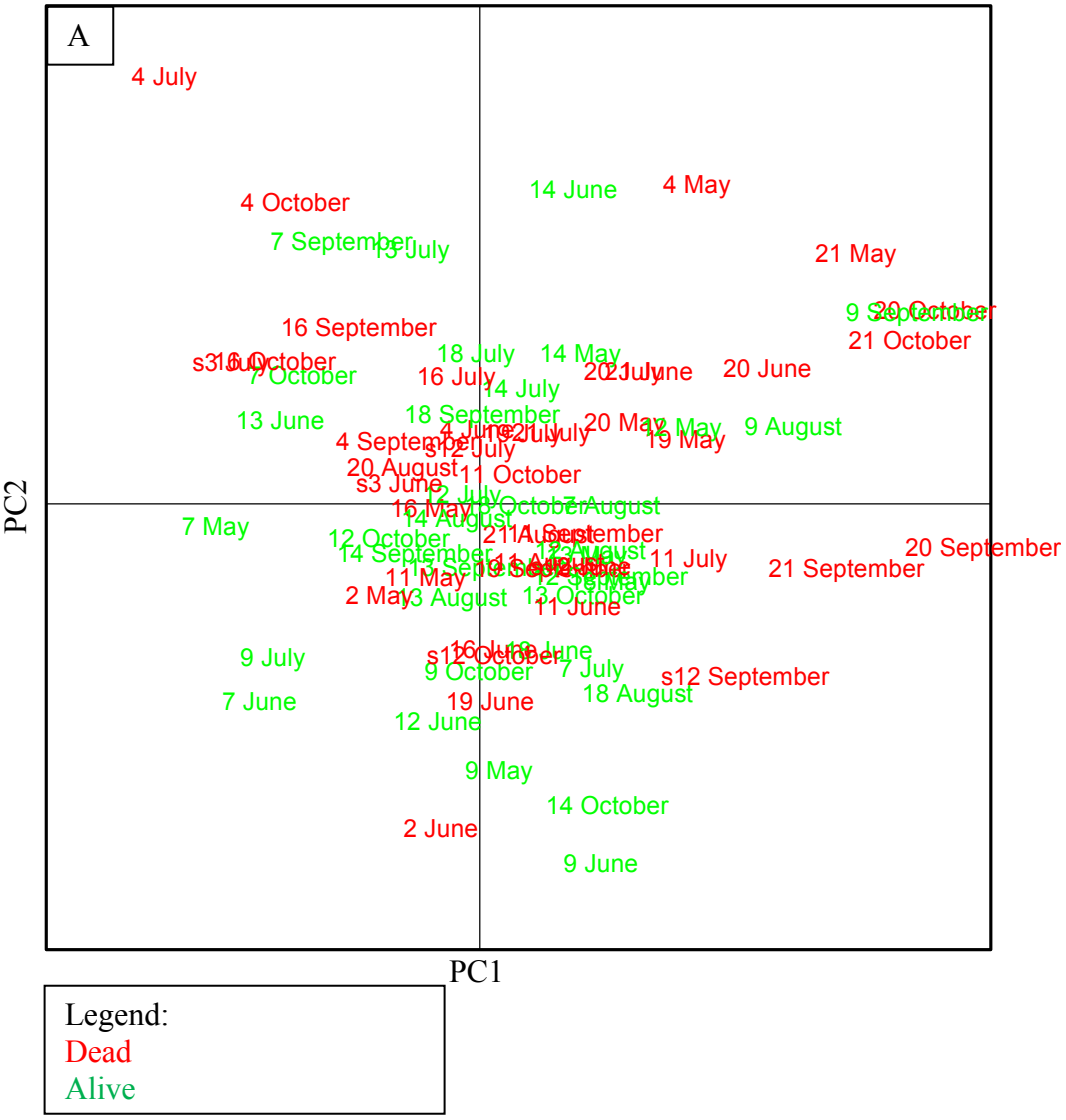
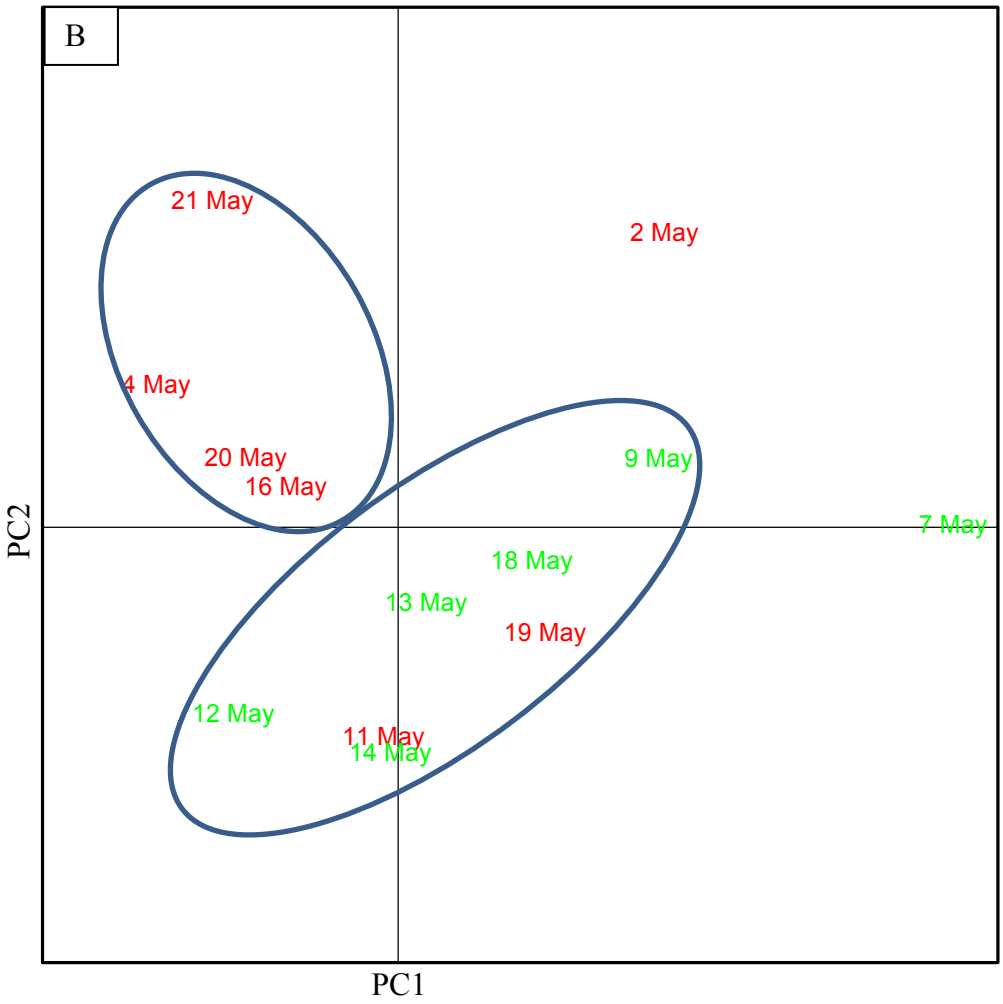
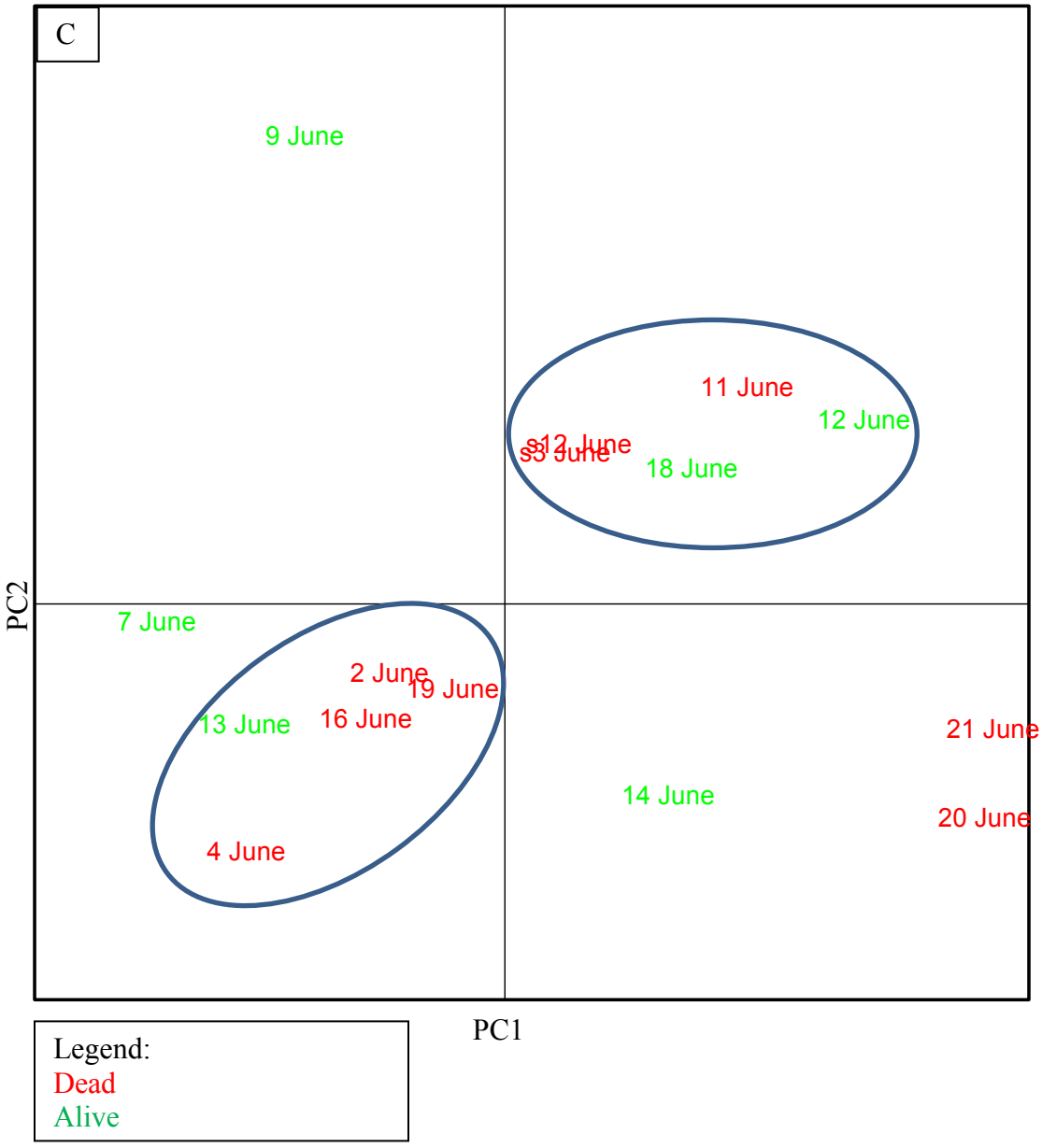


Figure 9: Average number of bands in bee bread samples observed by DGGE per month. Bands in which an observable peak was seen on the desitometric curve in the GelCompar II® software program were considered "prominent" and were included in the average. Bars represent SEM.

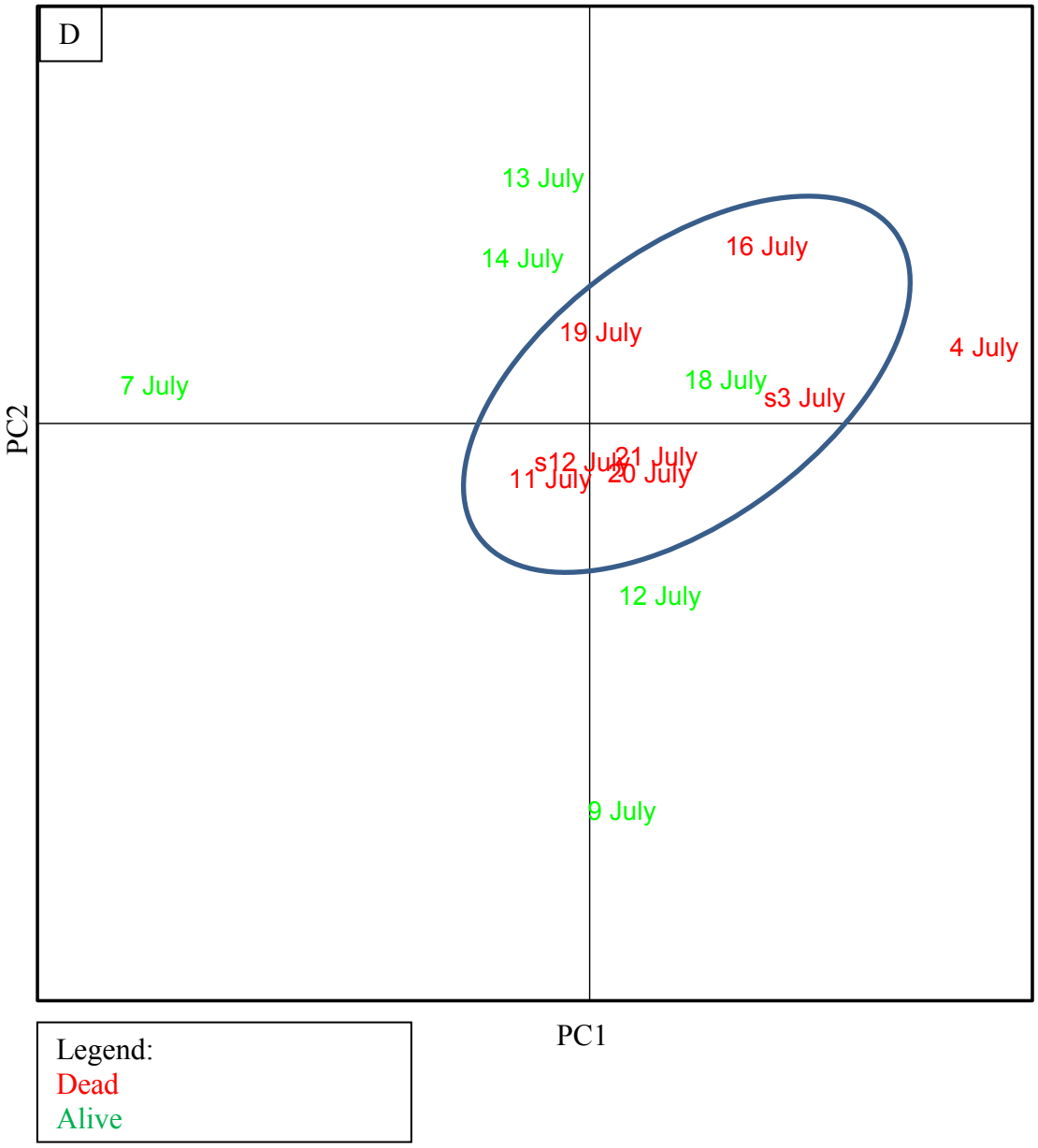

A
4 July
4 October
7 September
13 July
14 June
4 May
21 May
16 September
21 October
18 July
14 May
16 July
14 July
20 June
13 June
18 September
20 May
9 August
19 May
4 September
20 August
11 October
s3 June
16 May
14 August
7 May
12 October
11 July
21 September
20 September
11 May
2 May
3 August
11 June
9 July
9 October
7 July
s12 September
7 June
19 June
18 August
12 June
9 May
14 October
2 June
9 June
PC2
PC1
Legend:
Dead
Alive

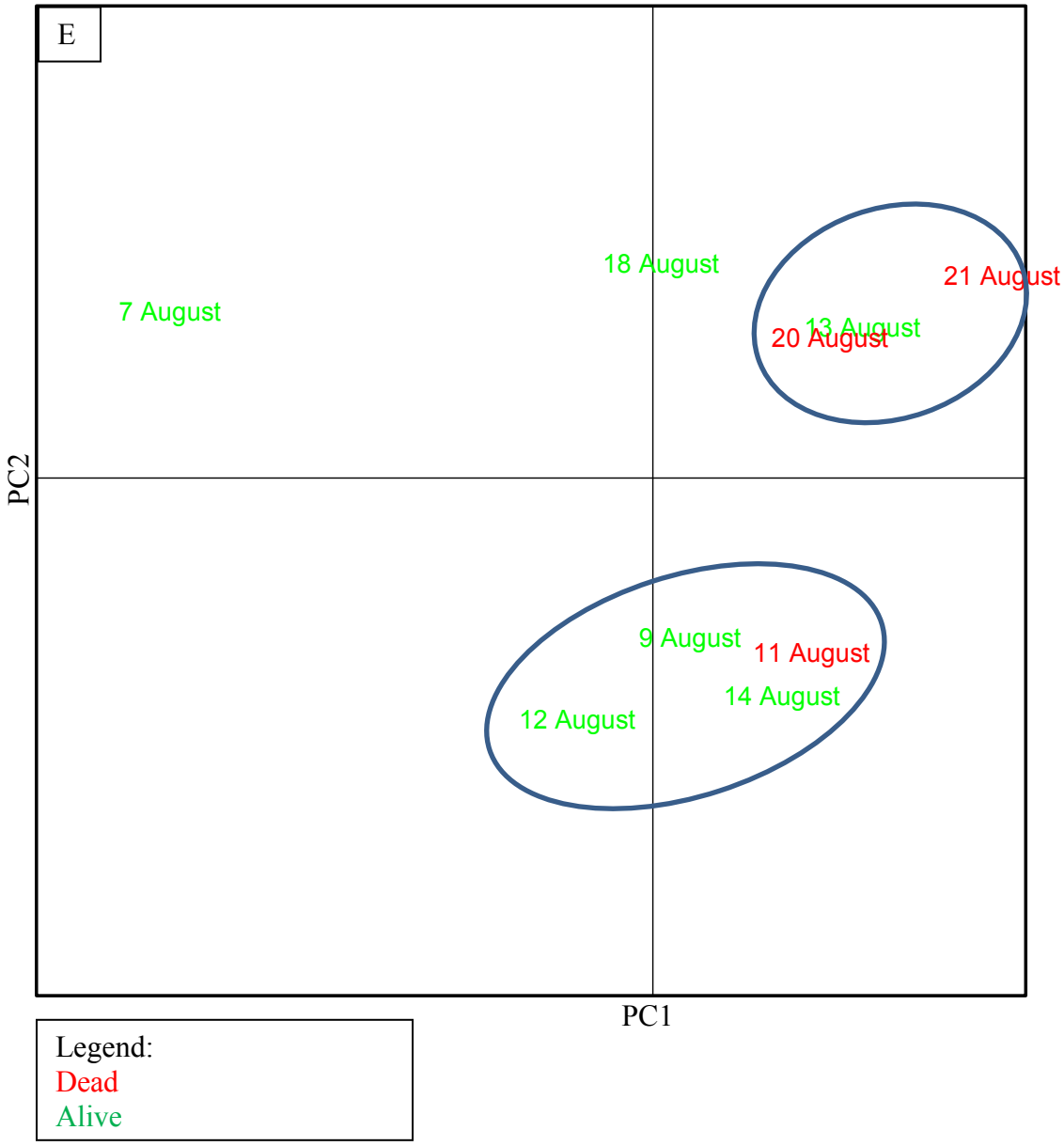


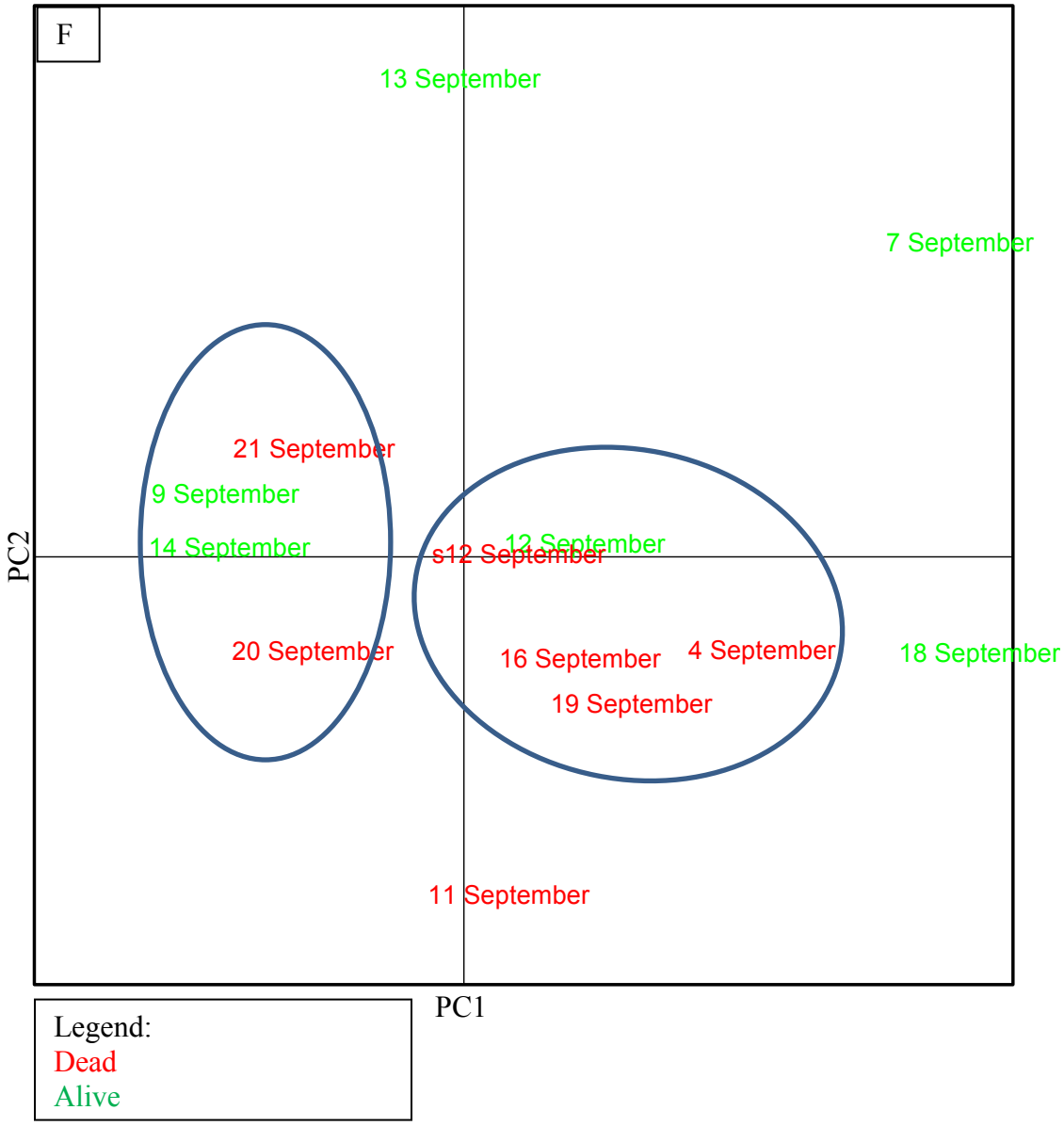
Legend:
Dead
Alive

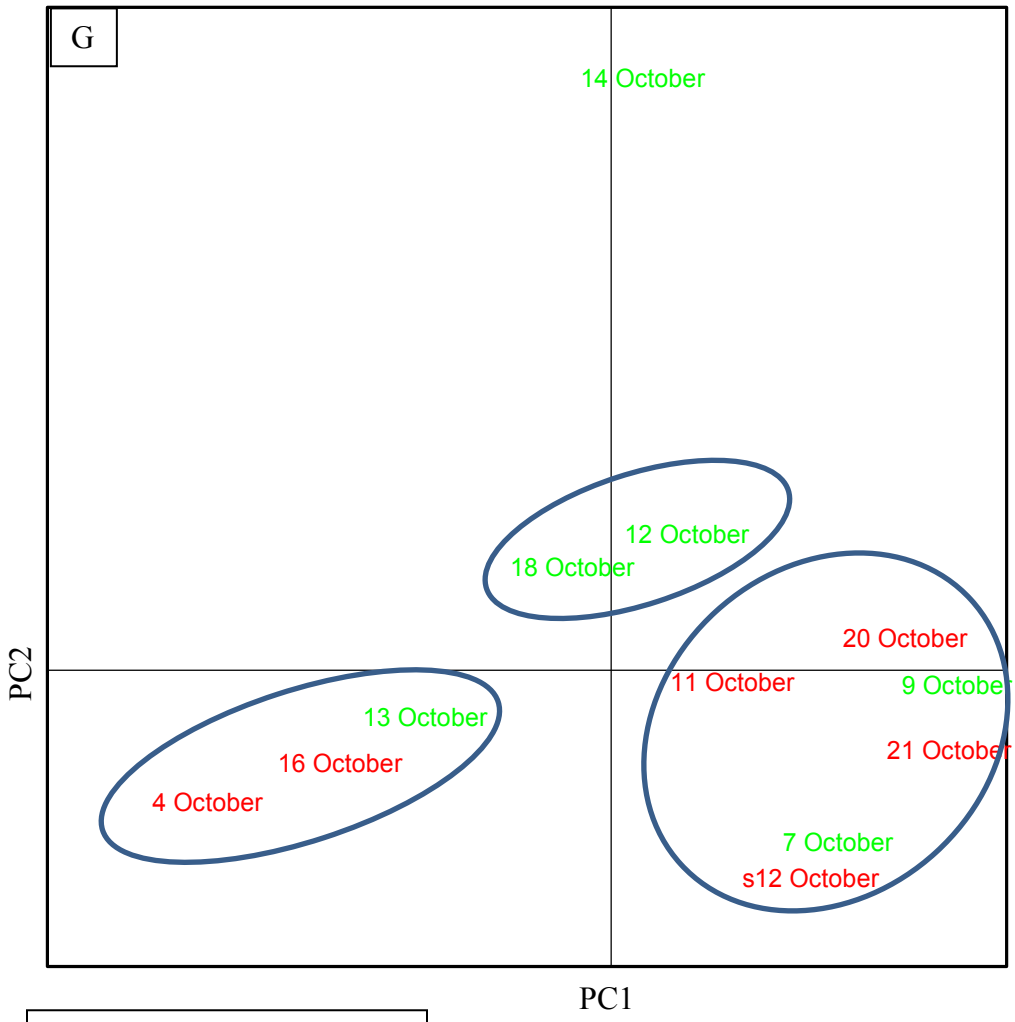

C
9 June
11 June
12 June
13 June
3 June
18 June
7 June
2 June
19 June
13 June
16 June
21 June
14 June
20 June
4 June
PC2
PC1
Legend:
Dead
Alive

`


D
13 July
14 July
16 July
19 July
4 July
7 July
18 July
s3 July
s12 July
21 July
11 July
20 July
12 July
9 July
PC2
PC1
Legend:
Dead
Alive

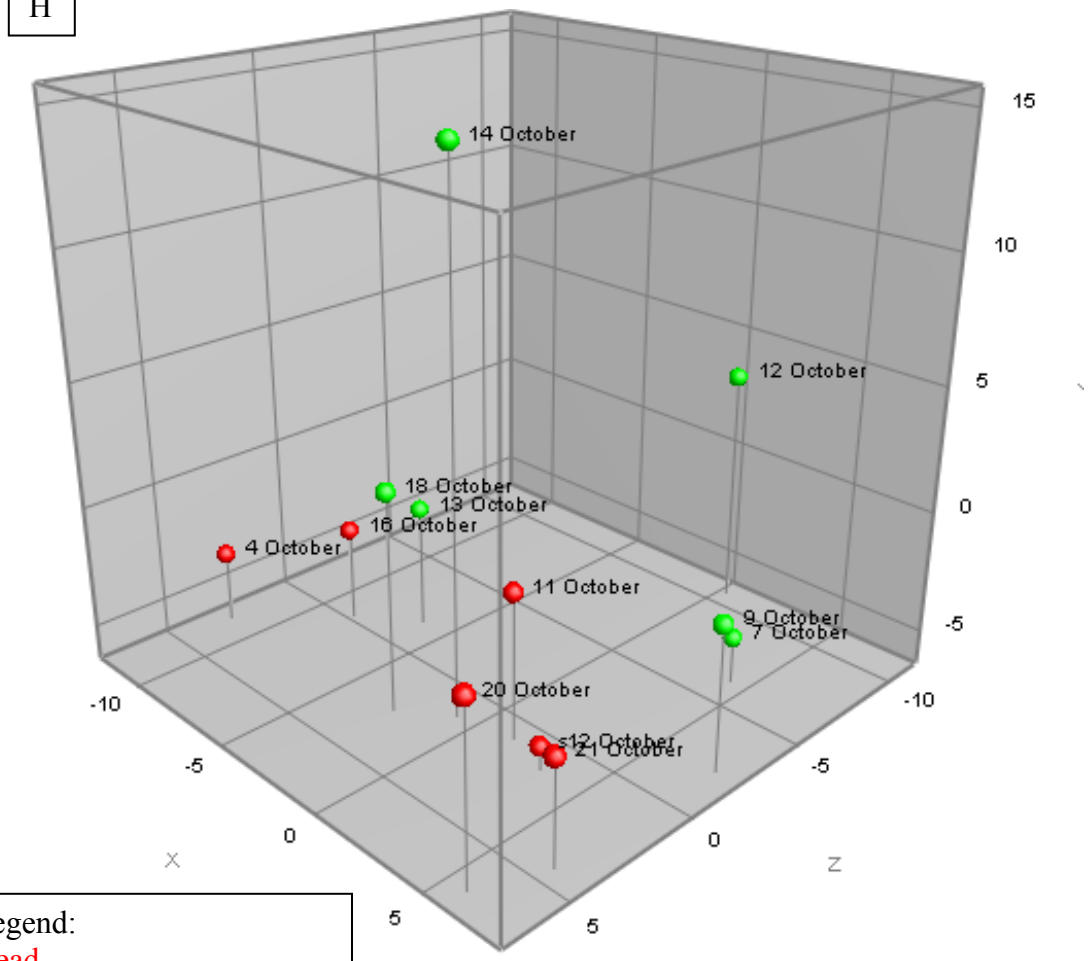

E
18 August
21 August
7 August
13 August
20 August
9 August
11 August
14 August
12 August
PC2
PC1
Legend:
Dead
Alive


F
13 September
7 September
21 September
9 September
14 September
12 September
s12 September
20 September
16 September
4 September
18 September
19 September
11 September
PC2
PC1
Legend:
Dead
Alive



Legend:
Dead
Alive

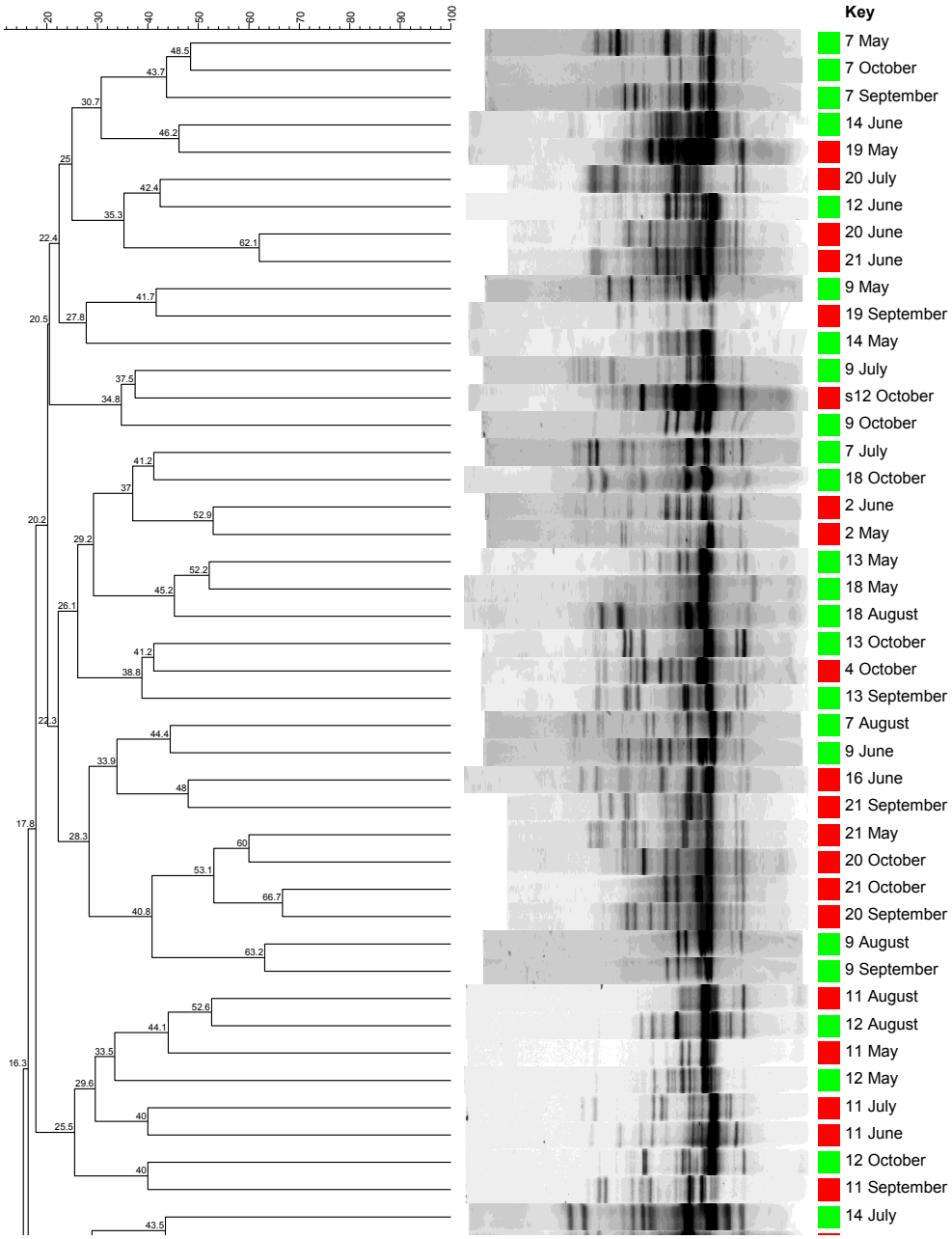
H



Legend:
Dead
Alive

Figure 10: PCA of bee bread bacterial profiles by colony survival in February 2014. Preceding numbers indicate which colony the bee bread was sampled from and the following month indicates the month the bee bread was sampled. Colonies hilighted green were still alive in February of 214. Colonies that are highlighted red were dead in February of 2014. A.) 2D PCA of all bee bread samples. The first and second components represent 5.4% and 5.0% of the variation respectively. B.) 2D PCA of May bee bread samples. The first and second components represent 16.8% and 12.8% of the variation respectively. C.) 2D PCA of June bee bread samples. The first and second components represent 15.2% and 11.3% of the variation respectively. D.) 2D PCA of July bee bread samples. The first and second components represent 14.9% and 12.8% of the variation respectively. E.) 2D PCA of August bee bread samples. The first and second components represent 19.4% and 16.5% of the variation respectively. F.) 2D PCA of September bee bread samples. The first and and second components represents 14.6% and 12.2% of the variation respectively. G and H.) 2D and 3D PCA October bee bread samples. The first, second, and third components represents 17.3%, 14.9%, and 13.3% of the variation respectively.

A


Key
7 May
7 October
7 September
14 June
19 May
20 July
12 June
20 June
21 June
9 May
19 September
14 May
9 July
s12 October
9 October
7 July
18 October
2 June
2 May
13 May
18 May
18 August
13 October
4 October
13 September
7 August
9 June
16 June
21 September
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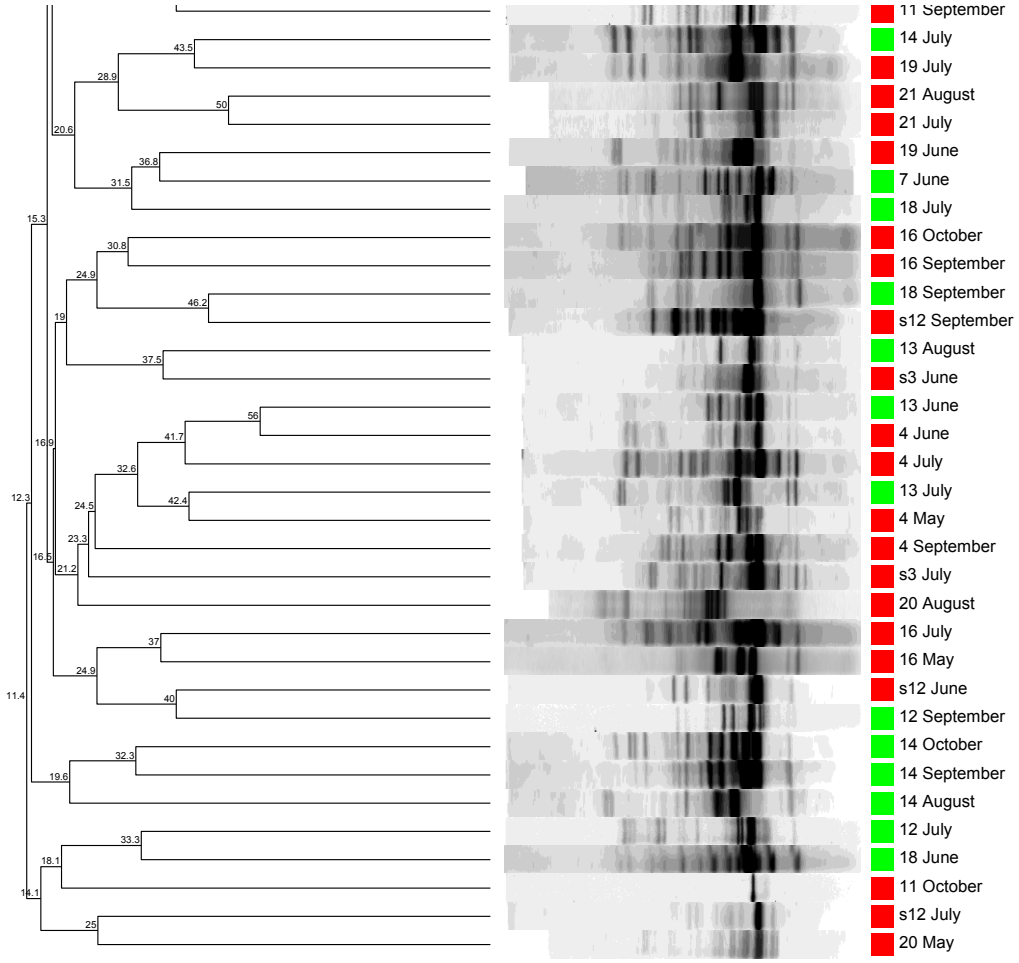
12 May
11 July
11 June
12 October
11 September
14 July


11 September
14 July
19 July
21 August
21 July
19 June
7 June
18 July
16 October
16 September
18 September
s12 September
13 August
s3 June
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4 June
4 July
13 July
4 May
4 September
s3 July
20 August
16 July
16 May
s12 June
12 September
14 October
14 September
14 August
12 July
18 June
11 October
s12 July
20 May
43.5
28.9
50
20.6
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31.5
15.3
30.8
24.9
46.2
19
37.5
56
41.7
16.9
32.6
12.3
42.4
24.5
23.3
16.5
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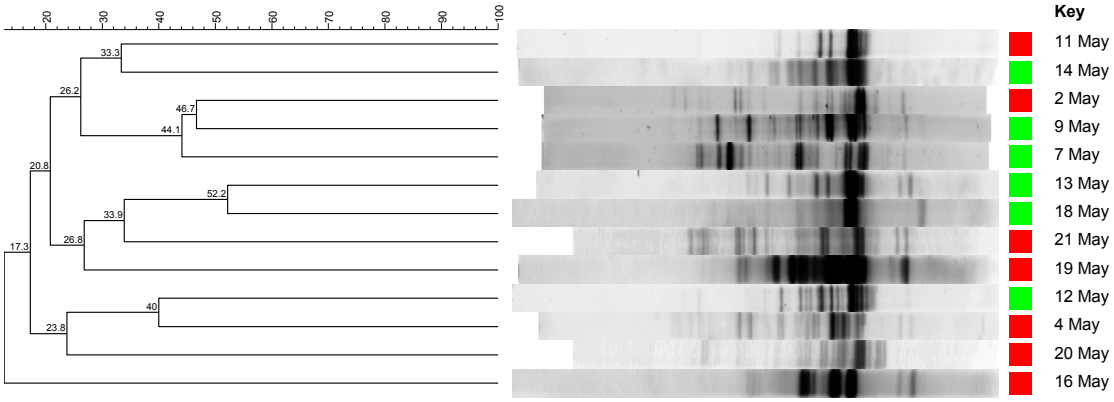
24.9
11.4
40
32.3
19.6
33.3
18.1
14.1
25

B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40
50
60
70
80
90
100
33.3
26.2
46.7
44.1
20.8
52.2
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17.3
26.8
40
23.8
Key
11 May
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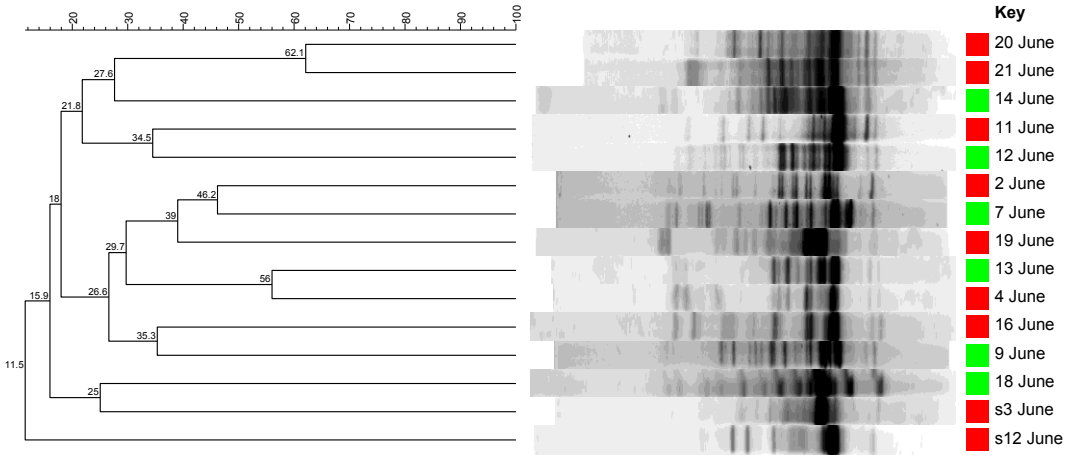
2 May
9 May
7 May
13 May
18 May
21 May
19 May
12 May
4 May
20 May
16 May

C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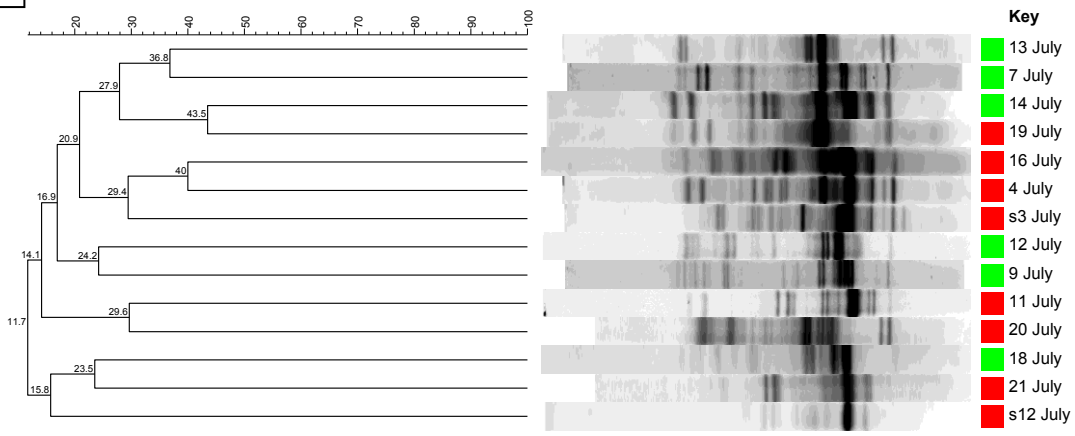
30
40
50
60
70
80
90
100
62.1
27.6
21.8
34.5
46.2
18
39
29.7
56
15.9
26.6
35.3
11.5
25
Key
20 June
21 June
14 June
11 June
12 June
2 June
7 June
19 June
13 June
4 June
16 June
9 June
18 June
s3 June
s12 June

D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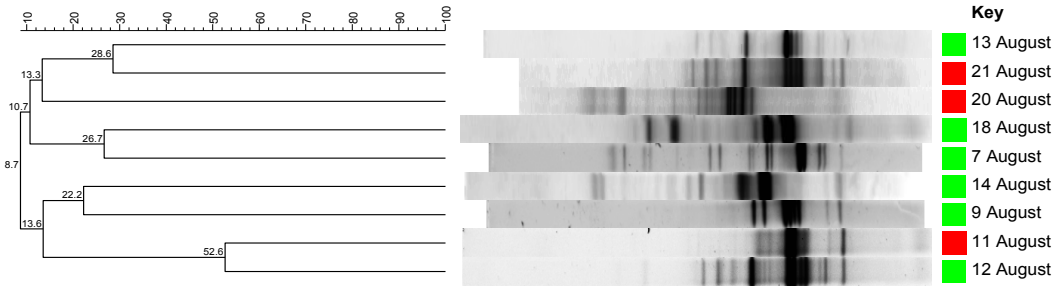
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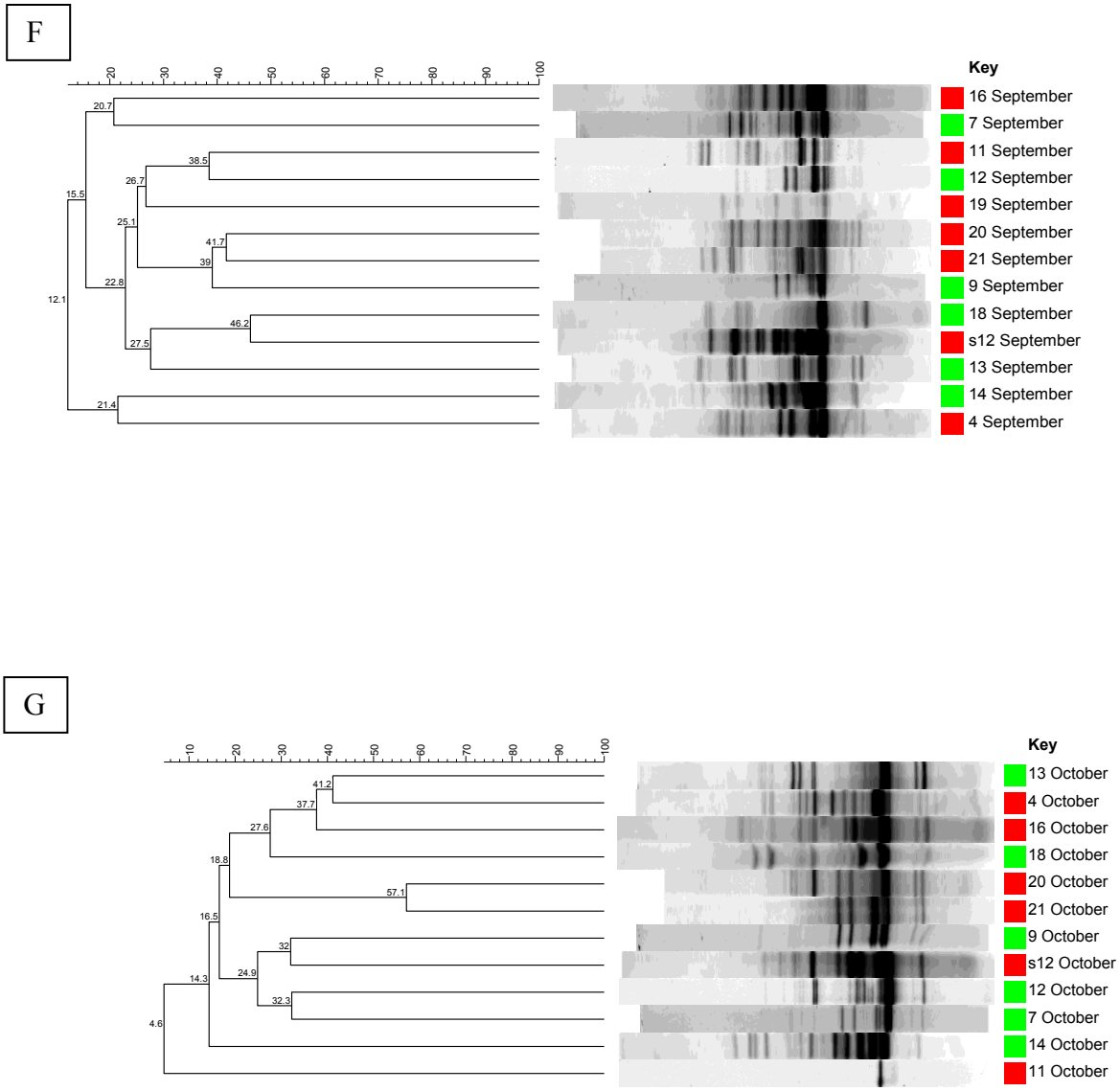
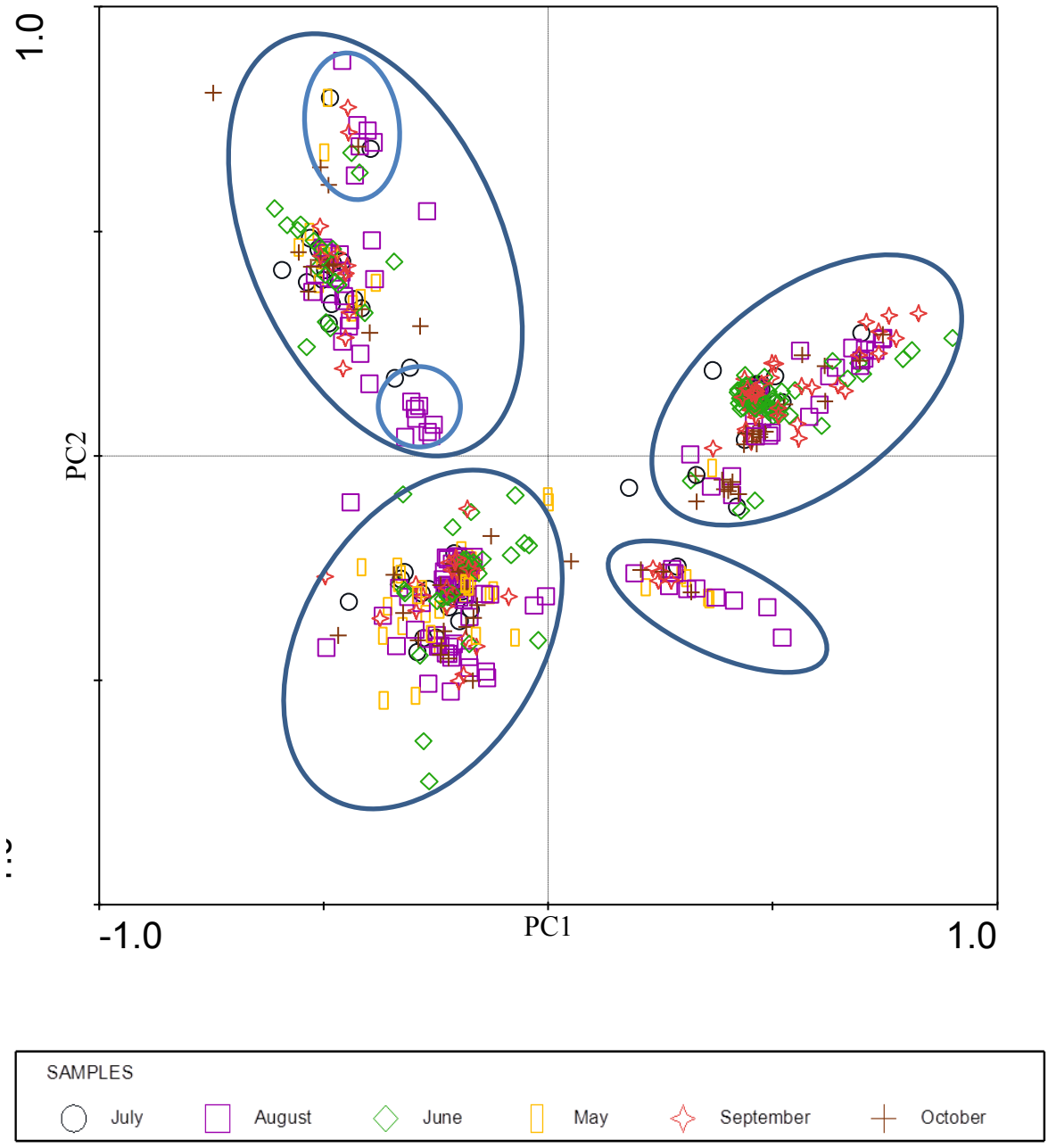


Figure 11: Hierarchical cluster analysis of bee bread bacterial banding patterns expressed as UPGMA dendrograms. The similarity is expressed as percent similarity (left). The bacterial profiles and the survival status of each colony are also shown (right). Colony names following red squares indicate the colony died during the winter. Colonies following green squares indicate the colony survived the winter. (A) Dendrogram of all samples (B) May samples, (C) June samples, (D) July samples, (E) August samples, (F) September samples, and (G) October samples.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SAMPLES
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Figure 12: Representation of all bacterial isolates tested by FAME analysis based on their fatty acid profiles by PCA. The first and second components represent 16.6% and 9.9% of the variation respectively. The upper left circle includes Gram-positive organisms predominantly of the *Bacillus* genus. The inner upper and lower circles were composed of *Bacillus mycoides* and *Staphylococcus xylosus* respectively. The upper left circle includes predominately Gram-negative organisms. The lower left circle includes predominately organisms that were not identified because their Sim index was below 0.600. The lower right circle includes organisms that had no match in the MIDI Sherlock® Microbial Identification System library.

REFERENCES

1. van Engelsdorp D, Steinhauer N, Rennich K, Pettis J, Lengerich EJ, Tarpy D, Delaplane KS, Spleen AM, Wilkes JT, Rose R, Lee K, Wilson M, Skinner J, Caron D. Winter Loss Survey 2012-2013: Preliminary Results2013.
2. DeGrandi-Hoffman G, Eckholm B, Anderson KE. Honey Bee Health: The Potential Role of Microbes: Taylor & Francis Group, LLC; 2012.
3. Anderson KE, Sheehan TH, Eckholm BJ, Mott BM, DeGrandi-Hoffman G. An emerging paradigm of colony health: microbial balance of the honey bee and hive (Apis mellifera). Insectes Sociaux. 2011;58(4):431-44. doi: 10.1007/s00040-011-0194-6. PubMed PMID: WOS:000294821000001.
4. Gilliam M. Microbiology of Pollen and Bee Bread - Genus Bacillus. Apidologie. 1979;10(3):269-74. doi: 10.1051/apido:19790304. PubMed PMID: WOS:A1979HV16400004.
5. Hitchcock JD, Moffett JO, Lackett JJ, Elliott JR. Tylosin for Control of American Foulbrood Disease In Honey Bees. Journal of Economic Entomology. 1970;63(1):204-&. PubMed PMID: WOS:A1970F468600055.
6. Ampicillin [cited 2014 May 31, 2014]. Available from: http://livertox.nlm.nih.gov/Ampicillin.htm.
7. Streptomycin and Streptomycin Sulfate. 1992.
8. Pettis JS, Feldlaufer MI. Efficacy of lincomycin and tylosin in controlling American foulbrood in honey bee colonies. Journal of Apicultural Research. 2005;44(3):106-8. PubMed PMID: WOS:000232290900003.
9. Suszkiw J. New Antibiotic Approved for Treating Bacterial Honey Bee Disease 2005 [cited 2014 February 2, 2014].
10. Levy SB, Marshall BM. Honeybees and Tetracycline Resistance. Mbio. 2013;4(1):2. doi: 10.1128/mBio.00045-13. PubMed PMID: WOS:000315814300006.
11. Huang WF, Solter LF, Yau PM, Imai BS. Nosema ceranae Escapes Fumagillin Control in Honey Bees. Plos Pathogens. 2013;9(3):9. doi: 10.1371/journal.ppat.1003185. PubMed PMID: WOS:000316953800004.
12. McKee BA, Goodman RD, Saywell C, Hepworth G. Oxytetracycline hydrochloride activity in honey bee larvae (Apis mellifera) following medication with various doses. Apidologie. 2003;34(3):269-79. doi: 10.1051/apido:2003018. PubMed PMID: WOS:000182653000008.
13. vanEngelsdorp D, Meixner MD. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. Journal of Invertebrate Pathology. 2010;103:S80-S95. doi: 10.1016/j.jip.2009.06.011. PubMed PMID: WOS:000273993100010.
14. Kevin H. Colony Collapse Disorder Action Plan. 2007.

15. Herbert EW. The Hive and the Honey Bee1975.
16. Caron D. Honey Bee Biology and Beekeeping. 2001.
17. Hackett K, Purcell-Miramontes M, Rose R, Stewart C, Dowdy A, Boess B, Jones L, Holy D, Epstein D, Steeger T, Moriarty T, Vaughan A. Colony Collapse Disorder 2012 Annual Progress Report. 2012.
18. Dainat B, Evans JD, Chen YP, Gauthier L, Neumann P. Predictive Markers of Honey Bee Colony Collapse. Plos One. 2012;7(2):9. doi: 10.1371/journal.pone.0032151. PubMed PMID: WOS:000302916100055.
19. Genersch E, Aubert M. Emerging and re-emerging viruses of the honey bee (Apis mellifera L.). Veterinary Research. 2010;41(6):20. doi: 10.1051/vetres/2010027. PubMed PMID: WOS:000285549500008.
20. Kluser S, Neumann P, Chuzat M-P, Pettis J, Vaissière B, Duthie D. UNEP Emerging Issues: Global Honey Bee Colony Disorder and Threats to Insect Pollinators. United Nations Environment Programme (UNEP), 2010.
21. vanEngelsdorp D, Steinhauer N, Rennich K, Pettis J, Lengerich EJ, Tarpy D, Delaplane KS, Spleen AM, Wilkes JT, Rose R, Lee K, Wilson M, Skinner J, Caron DM. Winter Loss Survey 2012-2013 Preliminary Results. 2013.
22. Gilliam M. Identification and roles of non-pathogenic microflora associated with honey bees. Fems Microbiology Letters. 1997;155(1):1-10. PubMed PMID: WOS:000071091300001.
23. Vasquez A, Olofsson TC. The lactic acid bacteria involved in the production of bee pollen and bee bread. Journal of Apicultural Research. 2009;48(3):189-95. doi: 10.3896/ibra.1.48.3.07. PubMed PMID: WOS:000267851900007.
24. Evans JD, Armstrong T-N. Antagonistic interactions between honey bee bacterial symbionts and implications for disease. BMC Ecology. 2006;6:9pp. PubMed PMID: ZOOREC:ZOOR14209059268.
25. Olofsson TC, Vasquez A. Detection and identification of a novel lactic acid bacterial flora within the honey stomach of the honeybee Apis mellifera. Current Microbiology. 2008;57(4):356-63. doi: 10.1007/s00284-008-9202-0. PubMed PMID: WOS:000258654500015.
26. Douglas AE. The microbial dimension in insect nutritional ecology. Functional Ecology. 2009;23(1):38-47. doi: 10.1111/j.1365-2435.2008.01442.x. PubMed PMID: WOS:000262510400005.
27. Haydak MH, Palmer LS. Royal jelly and bee bread as sources of vitamins B-1, B-2, B-6, C, and nicotinic and pantothenic acids. Journal of Economic Entomology. 1942;35(3):319-20. PubMed PMID: WOS:000201405300004.
28. DeGrandi-Hoffman G, Eckholm B, Huang MH. Concentrations of Nutrients in Pollen Can Change After Conversion to Bee Bread. American Bee Journal. 2013;153(11):1195-8. PubMed PMID: WOS:000331011900018.
29. Evans JD, Schwarz RS. Bees brought to their knees: microbes affecting honey bee health. Trends in Microbiology. 2011;19(12):614-20. doi: 10.1016/j.tim.2011.09.003. PubMed PMID: WOS:000299014300006.
30. Shimanuki H, Knox DA. Diagnosis of Honey Bee Diseases. 2000.

31. Berg RD. The indigenous gastrointestinal microflora. Trends in Microbiology. 1996;4(11):430-5. doi: 10.1016/0966-842x(96)10057-3. PubMed PMID: WOS:A1996VT27100009.
32. Loper GM, Standifer LN, Thompson MJ, Gilliam M. Biochemistry and Microbiology of Bee-Collected Almond (Prunus-Dulcis) Pollen and Bee Bread .1. Fatty-Acids, Sterols, Vitamins and Minerals. Apidologie. 1980;11(1):63-73. doi: 10.1051/apido:19800108. PubMed PMID: WOS:A1980KB96600008.
33. Connor LJ, Muir RG. Pollen and Bee Bread. American Bee Journal. 2013;153(7):727-9. PubMed PMID: WOS:000331011100012.
34. Anderson KE, Sheehan TH, Mott BM, Maes P, Snyder L, Schwan MR, Walton A, Jones BM, Corby-Harris V. Microbial Ecology of the Hive and Pollination Landscape: Bacterial Associates from Floral Nectar, the Alimentary Tract and Stored Food of Honey Bees (Apis mellifera). Plos One. 2013;8(12):16. doi: 10.1371/journal.pone.0083125. PubMed PMID: WOS:000328737700049.
35. Corby-Harris V, Maes P, Anderson KE. The Bacterial Communities Associated with Honey Bee (Apis mellifera) Foragers. PloS one. 2014;9(4):e95056. doi: 10.1371/journal.pone.0095056. PubMed PMID: MEDLINE:24740297.
36. DeGrandi-Hoffman G, Eckholm B, Anderson KE. Honey Bee Health: The Potential Roles of Microbes. Sammataro D, Yoder JA, editors: Taylor and Francis Group, LLC.
37. Hitchcoc.Jd, Moffett JO, Lackett JJ, Elliott JR. Tylosin for Control of American Foulbrood Disease in Honey Bees. Journal of Economic Entomology. 1970;63(1):204-&. PubMed PMID: WOS:A1970F468600055.
38. Tian BY, Fadhil NH, Powell JE, Kwong WK, Moran NA. Long-Term Exposure to Antibiotics Has Caused Accumulation of Resistance Determinants in the Gut Microbiota of Honeybees. Mbio. 2012;3(6):7. doi: 10.1128/mBio.00377-12. PubMed PMID: WOS:000313100700011.
39. Oxytetracycline [cited 2013 August 28]. Available from: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?sid=46507025#x332.
40. Chopra I, Roberts M. Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiology and Molecular Biology Reviews. 2001;65(2):232-+. doi: 10.1128/mmbr.65.2.232-260.2001. PubMed PMID: WOS:000169135200003.
41. Oxytetracycline 2005 [updated February 8, 2013; cited 2014 February 2, 2014]. Available from: http://www.drugbank.ca/drugs/DB00595.
42. Oxytetracycline 2013 [cited 2013 August 28th]. Available from: http://www.drugbank.ca/drugs/DB00595.
43. Vidaver AK. Uses of antimicrobials in plant agriculture. Clinical Infectious Diseases. 2002;34:S107-S10. doi: 10.1086/340247. PubMed PMID: WOS:000175621100007.
44. Streptomycin: National Library of Medicine; [cited 2014 May 25]. Available from: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?sid=46506845.

45. Streptomycin 2013 [cited 2014 May 25]. Available from: http://www.drugbank.ca/drugs/DB01082.
46. Streptomycin (Agri-Strep, Agrimycin) EPA Pesticide Fact Sheet 9/88 1988 [2014]May 25]. Available from: http://pmep.cce.cornell.edu/profiles/fung-nemat/febuconazole-sulfur/streptomycin/fung-prof-streptomycin.html.
47. Zhanel GG, Dueck M, Hoban DJ, Vercaigne LM, Embil JM, Gin AS, Karlowsky JA. Review of macrolides and ketolides - Focus on respiratory tract infections. Drugs. 2001;61(4):443-98. doi: 10.2165/00003495-200161040-00003. PubMed PMID: WOS:000168161100003.
48. Suchodolski JS, Dowd SE, Westermarck E, Steiner JM, Wolcott RD, Spillmann T, Harmoinen JA. The effect of the macrolide antibiotic tylosin on microbial diversity in the canine small intestine as demonstrated by massive parallel 16S rRNA gene sequencing. Bmc Microbiology. 2009;9:16. doi: 10.1186/1471-2180-9-210. PubMed PMID: WOS:000271287900001.
49. Westermarck E, Skrzypczak T, Harmoinen J, Steiner JM, Ruaux CG, Williams DA, Eerola E, Sundback P, Rinkinen M. Tylosin-responsive chronic diarrhea in dogs. Journal of Veterinary Internal Medicine. 2005;19(2):177-86. doi: 10.1892/0891-6640(2005)19<177:tcdid>2.0.co;2. PubMed PMID: WOS:000227882600008.
50. Klee J, Besana AM, Genersch E, Gisder S, Nanetti A, Tam DQ, Chinh TX, Puerta F, Ruz JM, Kryger P, Message D, Hatjina F, Korpela S, Fries I, Paxton RJ. Widespread dispersal of the microsporidian Nosema ceranae, an emergent pathogen of the western honey bee, Apis mellifera. Journal of Invertebrate Pathology. 2007;96(1):1-10. doi: 10.1016/j.jip.2007.02.014. PubMed PMID: WOS:000249058100001.
51. Arico-Muendel C, Centrella PA, Contonio BD, Morgan BA, O'Donovan G, Paradise CL, Skinner SR, Sluboski B, Svendsen JL, White KF, Debnath A, Gut J, Wilson N, McKerrow JH, DeRisi JL, Rosenthal PJ, Chiang PK. Antiparasitic activities of novel, orally available fumagillin analogs. Bioorganic & Medicinal Chemistry Letters. 2009;19(17):5128-31. doi: 10.1016/j.bmcl.2009.07.029. PubMed PMID: WOS:000268863800053.
52. Aminopenicillins: Third-Generation Penicillins [cited 2014 May 31, 2014]. Available from: http://livertox.nlm.nih.gov/Aminopenicillins.htm.
53. Tamaki H, Sekiguchi Y, Hanada S, Nakamura K, Nomura N, Matsumura M, Kamagata Y. Comparative analysis of bacterial diversity in freshwater sediment of a shallow eutrophic lake by molecular and improved cultivation-based techniques. Applied and Environmental Microbiology. 2005;71(4):2162-9. doi: 10.1128/aem.71.4.2162-2169.2005. PubMed PMID: WOS:000228338000064.
54. Nucleotide and oligotide composition of genomes: *all* University of the Basque Country; 2003-2013 [January 14, 2014]. Available from: http://insilico.ehu.es/oligoweb/index2.php?m=all.
55. Andrews ES. Analyzing Arthropods for the Presence of Bacteria. Current Protocols in Microbiology. Wiley Online Library: John Wiley & Sons, Inc; 2013. p. 1-4.

56. Korbie DJ, Mattick JS. Touchdown PCR for increased specificity and sensitivity in PCR amplification. Nature Protocols. 2008;3(9):1452-6. doi: 10.1038/nprot.2008.133. PubMed PMID: WOS:000266108100002.
57. Midi I. Sherlock Microbial Identification System Version 6.2. MIDI, Inc.; 2012. p. 2-14.
58. BD BBL™ Sensi-Disc™ Antimicrobial Susceptibility Test Discs. Becton, Dickinson and Company. p. 2-3.
59. Sun DL, Jiang X, Wu QLL, Zhou NY. Intragenomic Heterogeneity of 16S rRNA Genes Causes Overestimation of Prokaryotic Diversity. Applied and Environmental Microbiology. 2013;79(19):5962-9. doi: 10.1128/aem.01282-13. PubMed PMID: WOS:000324176900018.
60. Neilson JW, Jordan FL, Maier RM. Analysis of artifacts suggests DGGE should not be used for quantitative diversity analysis. Journal of Microbiological Methods. 2013;92(3):256-63. doi: 10.1016/j.mimet.2012.12.021. PubMed PMID: WOS:000316532600007.
61. Crosby LD, Criddle CS. Understanding bias in microbial community analysis techniques due to rrn operon copy number heterogeneity. Biotechniques. 2003;34(4):790-+. PubMed PMID: WOS:000182176800016.
62. Peixoto RS, Instituto de Microbiologia Professor Paulo de GÃ³es UFdRdJ, Rio de Janeiro,, Da Costa Coutinho HL, Embrapa Solos RdJ, Brazil,, Rumjanek NG, Embrapa Agrobiologia Sd, RJ, Brazil, Macrae A, Instituto de Microbiologia Professor Paulo de GÃ³es UFdRdJ, Rio de Janeiro,, Rosado AS, Instituto de Microbiologia Professor Paulo de GÃ³es UFdRdJ, Rio de Janeiro,. Use of rpoB and 16S rRNA genes to analyse bacterial diversity of a tropical soil using PCR and DGGE. Letters in Applied Microbiology. 2002;35(4):316-20. doi: 10.1046/j.1472-765X.2002.01183.x.
63. Dahllof I, Baillie H, Kjelleberg S. rpoB-based microbial community analysis avoids limitations inherent in 16S rRNA gene intraspecies heterogeneity. Applied and Environmental Microbiology. 2000;66(8):3376-80. doi: 10.1128/aem.66.8.3376-3380.2000. PubMed PMID: WOS:000088546300035.
64. Forsgren E, Olofsson TC, Vasquez A, Fries I. Novel lactic acid bacteria inhibiting Paenibacillus larvae in honey bee larvae. Apidologie. 2010;41(1):99-108. doi: 10.1051/apido/2009065. PubMed PMID: WOS:000273050600013.
65. Mattila HR, Rios D, Walker-Sperling VE, Roeselers G, Newton ILG. Characterization of the Active Microbiotas Associated with Honey Bees Reveals Healthier and Broader Communities when Colonies are Genetically Diverse. Plos One. 2012;7(3):11. doi: 10.1371/journal.pone.0032962. PubMed PMID: WOS:000302381500067.
66. Dainat B, Evans JD, Chen YP, Gauthier L, Neumann P. Dead or Alive: Deformed Wing Virus and Varroa destructor Reduce the Life Span of Winter Honeybees. Applied and Environmental Microbiology. 2012;78(4):981-7. doi: 10.1128/aem.06537-11. PubMed PMID: WOS:000299918600010.
67. Yoshiyama M, Wu MH, Sugimura Y, Takaya N, Kimoto-Nira H, Suzuki C. Inhibition of Paenibacillus larvae by lactic acid bacteria isolated from fermented

materials. Journal of Invertebrate Pathology. 2013;112(1):62-7. doi: 10.1016/j.jip.2012.09.002. PubMed PMID: WOS:000313758600010.
68. vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, Frazier M, Frazier J, Cox-Foster D, Chen YP, Underwood R, Tarpy DR, Pettis JS. Colony Collapse Disorder: A Descriptive Study. Plos One. 2009;4(8):17. doi: 10.1371/journal.pone.0006481. PubMed PMID: WOS:000268637700007.
69. Gilliam M. Microbiology of Pollen and Bee Bread - The Yeasts. Apidologie. 1979;10(1):43-53. doi: 10.1051/apido:19790106. PubMed PMID: WOS:A1979GZ80800006.
70. Vasquez A, Olofsson TC, Sammataro D. A scientific note on the lactic acid bacterial flora in honeybees in the USA - A comparison with bees from Sweden. Apidologie. 2009;40(1):26-8. doi: 10.1051/apido:2008063. PubMed PMID: WOS:000263384900004.
71. Olofsson TC, Vasquez A, Sammataro D, Macharia J. A scientific note on the lactic acid bacterial flora within the honeybee subspecies Apis mellifera (Buckfast), A. m. scutellata, A. m. mellifera, and A. m. monticola. Apidologie. 2011;42(6):696-9. doi: 10.1007/s13592-011-0064-2. PubMed PMID: WOS:000297707400003.
72. Hunt GJ, Page RE, Fondrk MK, Dullum CJ. Major Quantitative Trait Loci Affecting Honey-Bee Foraging Behavior. Genetics. 1995;141(4):1537-45. PubMed PMID: WOS:A1995TH41900025.
73. Kuhnholz S, Seeley TD. The control of water collection in honey bee colonies. Behavioral Ecology and Sociobiology. 1997;41(6):407-22. doi: 10.1007/s002650050402. PubMed PMID: WOS:000071149000006.
74. Gilliam M, Valentin.Dk. Enterobacteriaceae Isolated from Foraging Worker Honey Bees, Apis-Mellifera. Journal of Invertebrate Pathology. 1974;23(1):38-41. doi: 10.1016/0022-2011(74)90069-x. PubMed PMID: WOS:A1974S355900005.
75. Mongodin EF, Shapir N, Daugherty SC, Deboy RT, Emerson JB, Shvartzbeyn A, Radune D, Vamathevan J, Riggs F, Grinberg V, Khouri H, Wackett LP, Nelson KE, Sadowsky MJ. Secrets of soil survival revealed by the genome sequence of Arthrobacter aurescens TC1. Plos Genetics. 2006;2(12):2094-106. doi: 10.1371/journal.pgen.0020214. PubMed PMID: WOS:000243482100013.
76. Drobniewski FA. Bacillus-Cereus and Related Species. Clinical Microbiology Reviews. 1993;6(4):324-38. PubMed PMID: WOS:A1993MF02500002.
77. Panda AK, Bisht SS, DeMondal S, Kumar NS, Gurusubramanian G, Panigrahi AK. Brevibacillus as a biological tool: a short review. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology. 2014;105(4):623-39. doi: 10.1007/s10482-013-0099-7. PubMed PMID: WOS:000332794200001.
78. Brenner DJ, Krieg NR, Staley JT. Bergy's Manual of Systematic Bacteriology. 2 ed. Garrity GM, editor. New York, New York: Springer Science and Business Media, Inc.; 1984.
79. Panasiti V, Devirgiliis V, Mancini M, Curzio M, Rossi M, Fioriti D, Pietropaolo V, Nicosia R, Gallinelli C, Chiarini F, Pecorini G, Calvieri S. A cutaneous infection caused by Brevundimonas vesicularis: A case report. International Journal of

Immunopathology and Pharmacology. 2008;21(2):457-61. PubMed PMID: WOS:000257576900026.
80. Chang S-C. Citrobacter species 2011 [cited 2014 June 23, 2014]. Available from: http://www.antimicrobe.org/b93.asp#r48.
81. Badger JL, Stins MF, Kim KS. Citrobacter freundii invades and replicates in human brain microvascular endothelial cells. Infection and Immunity. 1999;67(8):4208-15. PubMed PMID: WOS:000081637400067.
82. Grimont F, Griniont PAD. The Genus Enterobacter. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E, editors. Prokaryotes: a Handbook on the Biology of Bacteria, Vol 6, Third Edition: Proteobacteria: Gamma Subclass. New York: Springer; 2006. p. 197-214.
83. Ozkirim A. Seasonal Microflora, Especially Winter and Spring: Taylor & Francis Group, LLC; 2012.
84. Livermore DM. Mechanisms of Resistance to Cephalosporin Antibiotics. Drugs. 1987;34:64-88. doi: 10.2165/00003495-198700342-00007. PubMed PMID: WOS:A1987L003100006.
85. Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Oh MD, Kim EC, Choe KW. Bloodstream infections caused by Enterobacter species: Predictors of 30-day mortality rate and impact of broad-spectrum cephalosporin resistance on outcome. Clinical Infectious Diseases. 2004;39(6):812-8. doi: 10.1086/423382. PubMed PMID: WOS:000227491500010.
86. Townsend SM, Hurrell E, Caubilla-Barron J, Loc-Carrillo C, Forsythe SJ. Characterization of an extended-spectrum beta-lactamase Enterobacter hormaechei nosocomial outbreak, and other Enterobacter hormaechei misidentified as Cronobacter (Enterobacter) sakazakii. Microbiology-Sgm. 2008;154:3659-67. doi: 10.1099/mic.0.2008/021980-0. PubMed PMID: WOS:000261974700005.
87. Fisher K, Phillips C. The ecology, epidemiology and virulence of Enterococcus. Microbiology-Sgm. 2009;155:1749-57. doi: 10.1099/mic.0.026385-0. PubMed PMID: WOS:000267255200001.
88. Deletoile A, Decre D, Courant S, Passet V, Audo J, Grimont P, Arlet G, Brisse S. Phylogeny and Identification of Pantoea Species and Typing of Pantoea agglomerans Strains by Multilocus Gene Sequencing. Journal of Clinical Microbiology. 2009;47(2):300-10. doi: 10.1128/jcm.01916-08. PubMed PMID: WOS:000263029200003.
89. Coutinho TA, Venter SN. Pantoea ananatis: an unconventional plant pathogen. Molecular Plant Pathology. 2009;10(3):325-35. doi: 10.1111/j.1364-3703.2009.00542.x. PubMed PMID: WOS:000265229000002.
90. Cruz AT, Cazacu AC, Allen CH. Pantoea agglomerans, a plant pathogen causing human disease. Journal of Clinical Microbiology. 2007;45(6):1989-92. doi: 10.1128/jcm.00632-07. PubMed PMID: WOS:000247286500052.
91. Manos J, Belas R. The Genera Proteus, Providencia, and Morganella. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E, editors.

Prokaryotes: a Handbook on the Biology of Bacteria, Vol 6, Third Edition: Proteobacteria: Gamma Subclass. New York: Springer; 2006. p. 245-69.
92. Ozen AI, Ussery DW. Defining the Pseudomonas Genus: Where Do We Draw the Line with Azotobacter? Microbial Ecology. 2012;63(2):239-48. doi: 10.1007/s00248-011-9914-8. PubMed PMID: WOS:000300316500001.
93. Lister PD, Wolter DJ, Hanson ND. Antibacterial-Resistant Pseudomonas aeruginosa: Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms. Clinical Microbiology Reviews. 2009;22(4):582-+. doi: 10.1128/cmr.00040-09. PubMed PMID: WOS:000270711700004.
94. Thomson SV, Hansen DR, Flint KM. Dissemination of Bacteria Antagonistic to Erwinia-Amylovora by Honey-Bees. Plant Disease. 1992;76(10):1052-6. PubMed PMID: WOS:A1992JP10800021.
95. Hsueh PR, Teng LJ, Pan HJ, Chen YC, Sun CC, Ho SW, Luh KT. Outbreak of Pseudomonas fluorescens bacteremia among oncology patients. Journal of Clinical Microbiology. 1998;36(10):2914-7. PubMed PMID: WOS:000075896800020.
96. Bell KS, Philp JC, Aw DWJ, Christofi N. A review - The genus Rhodococcus. Journal of Applied Microbiology. 1998;85(2):195-210. PubMed PMID: WOS:000075625000001.
97. Grimont F, Grimont PAD. The Genus Serratia. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E, editors. Prokaryotes: a Handbook on the Biology of Bacteria, Vol 6, Third Edition: Proteobacteria: Gamma Subclass. New York: Springer; 2006. p. 219-44.
98. Bergy DH, Holt JG. Bergy's Manual of Determinative Bacteriology. Baltimore: Williams and Wilkins; 1994.
99. Ashelford KE, Fry JC, Bailey MJ, Day MJ. Characterization of Serratia isolates from soil, ecological implications and transfer of Serratia proteamaculans subsp quinovora Grimont et al 1983 to Serratia quinivorans corrig., sp nov. International Journal of Systematic and Evolutionary Microbiology. 2002;52:2281-9. doi: 10.1099/ijs.0.02263-0. PubMed PMID: WOS:000179838100051.
100. Czajkowski R, van der Wolf JM. Draft Genome Sequence of the Biocontrol Strain Serratia plymuthica A30, Isolated from Rotting Potato Tuber Tissue. Journal of Bacteriology. 2012;194(24):6999-7000. doi: 10.1128/jb.01699-12. PubMed PMID: WOS:000311922800074.
101. White DC, Sutton SD, Ringelberg DB. The genus Sphingomonas: Physiology and ecology. Current Opinion in Biotechnology. 1996;7(3):301-6. doi: 10.1016/s0958-1669(96)80034-6. PubMed PMID: WOS:A1996UT52600010.
102. Kersters K, Lisdiyanti P, Komagata K, Swings J. The Family Acetobacteraceae: The Genera Acetobacter, Acidomonas, Asaia, Gluconacetobacter, Gluconobacter, and Kozakia. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E, editors. Prokaryotes: a Handbook on the Biology of Bacteria, Vol 5, Third Edition: Proteobacteria: Alpha and Beta Subclasses. New York: Springer; 2006. p. 163-200.

103. Lim JA, Jee S, Lee DH, Roh E, Jung K, Oh C, Heu S. Biocontrol of Pectobacterium carotovorum subsp carotovorum Using Bacteriophage PP1. Journal of Microbiology and Biotechnology. 2013;23(8):1147-53. doi: 10.4014/jmb.1304.04001. PubMed PMID: WOS:000323687200014.
104. Park TH, Choi BS, Choi AY, Choi IY, Heu S, Park BS. Genome Sequence of Pectobacterium carotovorum subsp carotovorum Strain PCC21, a Pathogen Causing Soft Rot in Chinese Cabbage. Journal of Bacteriology. 2012;194(22):6345-6. doi: 10.1128/jb.01583-12. PubMed PMID: WOS:000310589300060.
105. Stothard P, Van Domselaar G, Shrivastava S, Guo A, O'Neill B, Cruz J, Ellison M, Wishart DS. BacMap: an interactive picture atlas of annotated bacterial genomes. Nucleic Acids Research. 2005;33:D317-D20.
106. Jyoti JL, Brewer GJ. Honey bees (Hymenoptera : Apidae) as vectors of Bacillus thuringiensis for control of branded sunflower moth (Lepidoptera : Tortricidae). Environmental Entomology. 1999;28(6):1172-6. PubMed PMID: WOS:000084592500035.
107. Yu H, Sutton JC. Effectiveness of bumblebees and honeybees for delivering inoculum of Gliocladium roseum to raspberry flowers to control Botrytis cinerea. Biological Control. 1997;10(2):113-22. doi: 10.1006/bcon.1997.0562. PubMed PMID: WOS:A1997XZ72700006.
108. Gray SM, Banerjee N. Mechanisms of arthropod transmission of plant and animal viruses. Microbiology and Molecular Biology Reviews. 1999;63(1):128-+. PubMed PMID: WOS:000078999400005.
109. Killiny N, Rashed A, Almeida RPP. Disrupting the Transmission of a Vector-Borne Plant Pathogen. Applied and Environmental Microbiology. 2012;78(3):638-43. doi: 10.1128/aem.06996-11. PubMed PMID: WOS:000299594200004.
110. Nault LR. Arthropod transmission of plant viruses: A new synthesis. Annals of the Entomological Society of America. 1997;90(5):521-41. PubMed PMID: WOS:A1997YH00300001.
111. Timmusk S, Grantcharova N, Wagner EGH. Paenibacillus polymyxa invades plant roots and forms biofilms. Applied and Environmental Microbiology. 2005;71(11):7292-300. doi: 10.1128/aem.71.11.7292-7300.2005. PubMed PMID: WOS:000233225000102.
112. Savini V, Catavitello C, Masciarelli G, Astolfi D, Balbinot A, Bianco A, Febbo F, D'Amario C, D'Antonio D. Drug sensitivity and clinical impact of members of the genus Kocuria. Journal of Medical Microbiology. 2010;59(12):1395-402. doi: 10.1099/jmm.0021709-0. PubMed PMID: WOS:000285271900001.
113. Wilson M. Bacteriology of humans: and ecological perspective. Malden, MA: Blackwell Pub.; 2008. p. 75.
114. Kocur M, Kloos WE, Schleifer KH. The Genus Micrococcus. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E, editors. Prokaryotes: a Handbook on the Biology of Bacteria, Vol 3, Third Edition: Archaea Bacteria: Firmicutes, Actinomycetes. New York: Springer; 2006. p. 961-71.

115. Chiller K, Selkin BA, Murakawa GJ. Skin microflora and bacterial infections of the skin. Journal of Investigative Dermatology Symposium Proceedings. 2001;6(3):170-4. doi: 10.1046/j.0022-202x.2001.00043.x. PubMed PMID: WOS:000174519000003.
116. Wray C, Wray A. Salmonella in Domestic Animals. Wallingford, Oxon, UK: CABI Pub.; 2000.
117. Coburn B, Grassl GA, Finlay BB. Salmonella, the host and disease: a brief review. Immunology and Cell Biology. 2007;85(2):112-8. doi: 10.1038/sj.icb.7100007. PubMed PMID: WOS:000245102500007.
118. Ismaael TG, Zamora EM, Khasawneh FA. Cedecea davisae's Role in a Polymicrobial Lung Infection in a Cystic Fibrosis Patient. Case reports in infectious diseases. 2012;2012:176864. doi: 10.1155/2012/176864. PubMed PMID: MEDLINE:23320209.
119. Perkins SR, Beckett TA, Bump CM. Cedecea-Davisae Bacteremia. Journal of Clinical Microbiology. 1986;24(4):675-6. PubMed PMID: WOS:A1986E113700042.
120. Welch RA. The Genus Escherichia. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E, editors. Prokaryotes: a Handbook on the Biology of Bacteria, Vol 6, Third Edition: Proteobacteria: Gamma Subclass. New York: Springer; 2006. p. 60-71.
121. Johnson TJ. Impacts of Fecal Bacteria on Human and Animal Health-Pathogens and Virulence Genes. Sadowsky MJ, Whitman RL, editors. Washington, DC: ASM Press; 2011.
122. Ryoo NH, Ha JS, Jeon DS, Kim JR, Kim HC. A case of pneumonia caused by Ewingella americana in a patient with chronic renal failure. Journal of Korean Medical Science. 2005;20(1):143-5. PubMed PMID: WOS:000228340600028.
123. Farmer JJ, Fanning GR, Huntleycarter GP, Holmes B, Hickman FW, Richard C, Brenner DJ. Kluyvera, A New (Redefined) Genus in The Family Enterobacteriaceae - Identification of Kluyvera-Ascorbata Sp-Nov And Kluyvera-Cryocrescens Sp-Nov In Clinical SPECIMENS. Journal of Clinical Microbiology. 1981;13(5):919-33. PubMed PMID: WOS:A1981LP14400025.
124. Pavan ME, Franco RJ, Rodriguez JM, Gadaleta P, Abbott SL, Janda JM, Zorzopulos J. Phylogenetic relationships of the genus Kluyvera: transfer of Enterobacter intermedius lzard et al. 1980 to the genus Kluyvera as Kluyvera intermedia comb. nov and reclassification of Kluyvera cochleae as a later synonym of K-intermedia. International Journal of Systematic and Evolutionary Microbiology. 2005;55:437-42. doi: 10.1099/ijs.0.63071-0. PubMed PMID: WOS:000226629100070.
125. Gneiding K, Frodl R, Funke G. Identities of Microbacterium spp. Encountered in Human Clinical Specimens. Journal of Clinical Microbiology. 2008;46(11):3646-52. doi: 10.1128/jcm.01202-08. PubMed PMID: WOS:000260532200015.
126. Liu J, Zhou Q, Ibrahim M, Liu H, Jin GL, Zhu B, Xie GL. Genome Sequence of the Biocontrol Agent Microbacterium barkeri Strain 2011-R4. Journal of

Bacteriology. 2012;194(23):6666-7. doi: 10.1128/jb.01468-12. PubMed PMID: WOS:000310924300064.

APPENDIX A

PCA OF ALL BEE BREAD BACTERIAL PROFILES BY MONTH

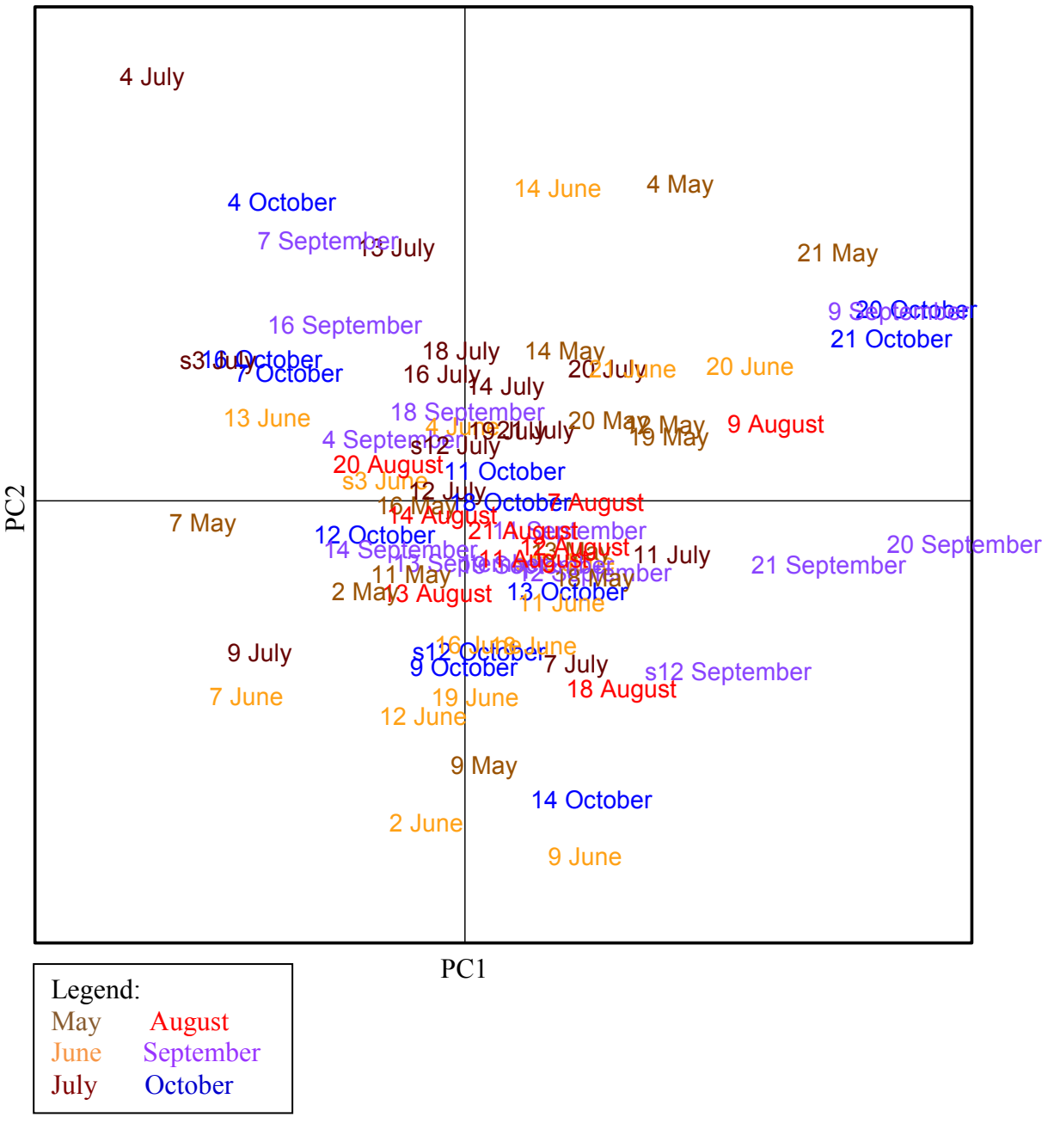
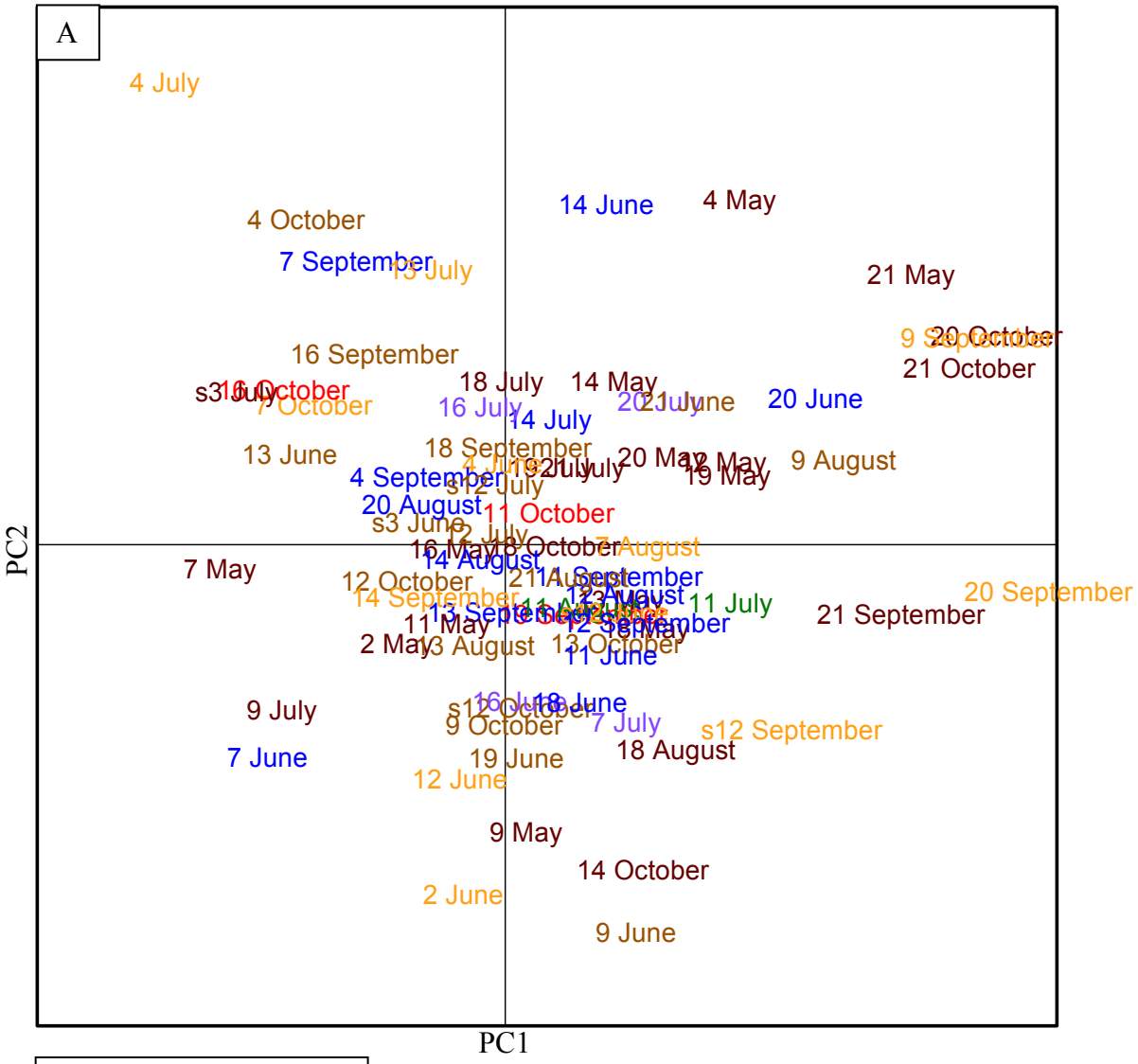


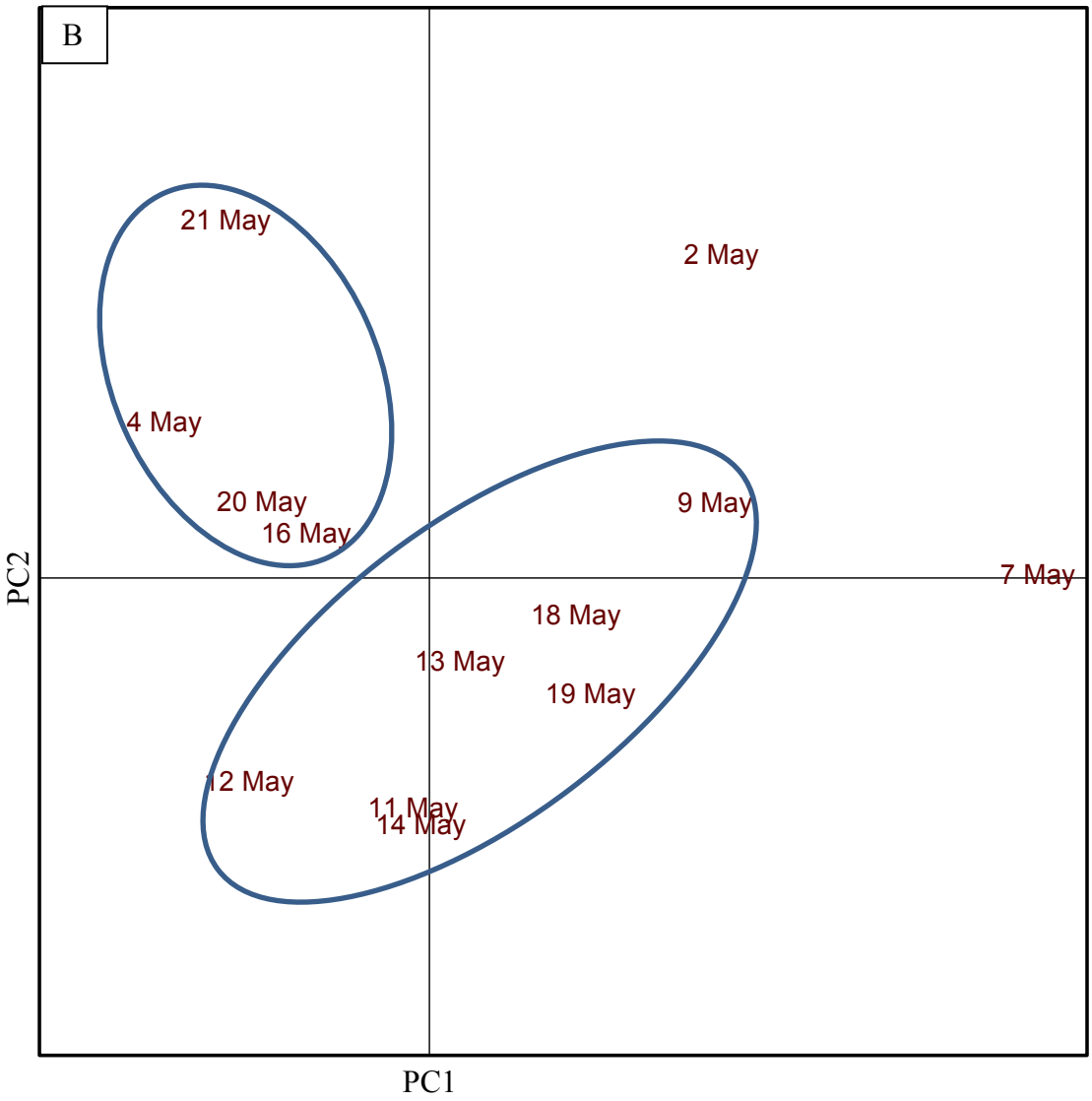
Figure A1: 2D PCA of all DGGE microbial profiles of bee bread sampled from the South Campus Research Apiary by month. Preceding numbers indicate which colony the bee bread was sampled from and the following month indicates the month the bee bread was sampled.The first and second component account for 5.4% and 5.0% of the variation respectively.

APPENDIX B

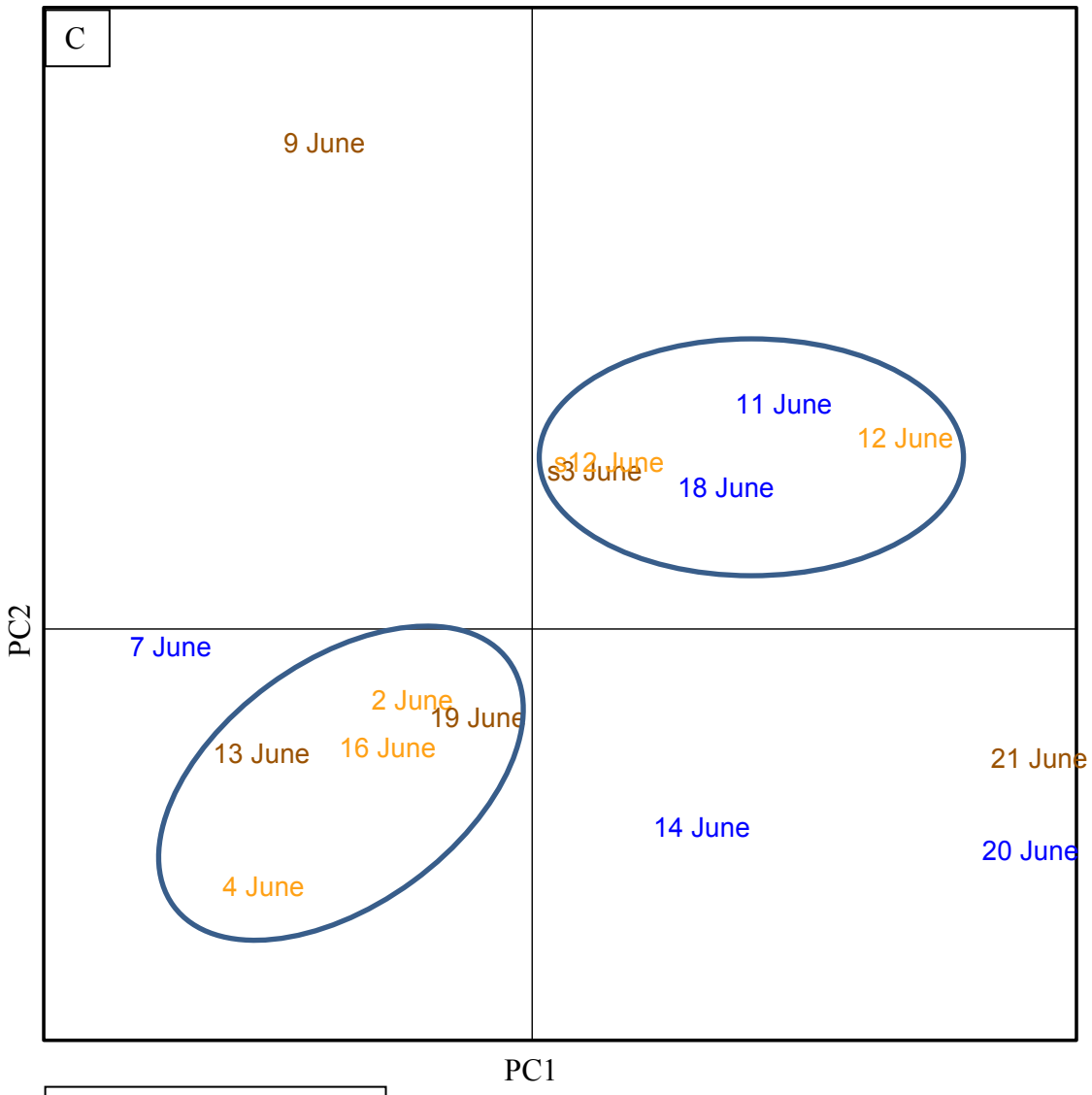
PCA OF ALL BEE BREAD BACTERIAL PROFILES BY THE NUMBER OF BEES



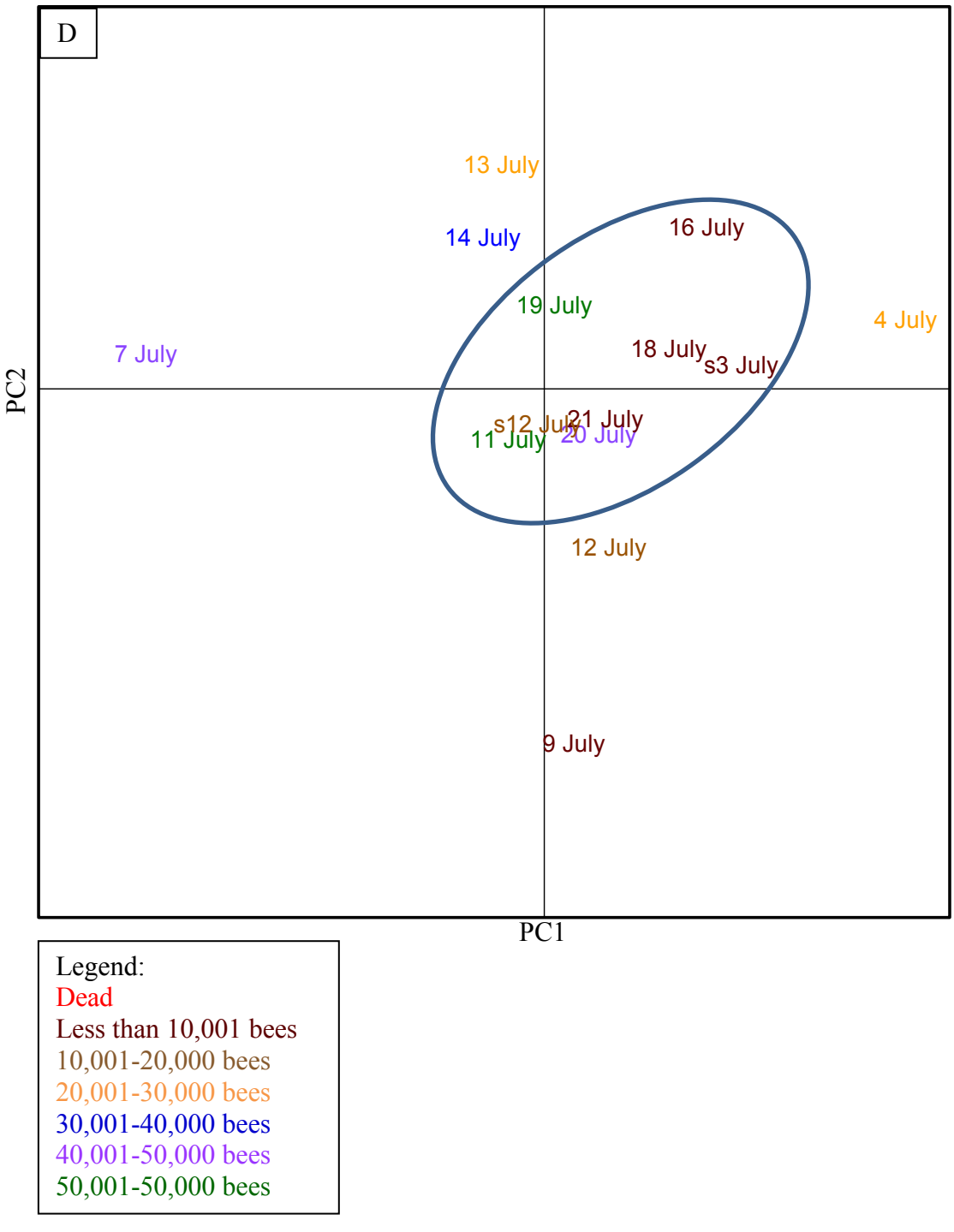
Legend:
Dead
Less than 10,001 bees
10,001-20,000 bees
20,001-30,000 bees
30,001-40,000 bees
40,001-50,000 bees
50,001-50,000 bees

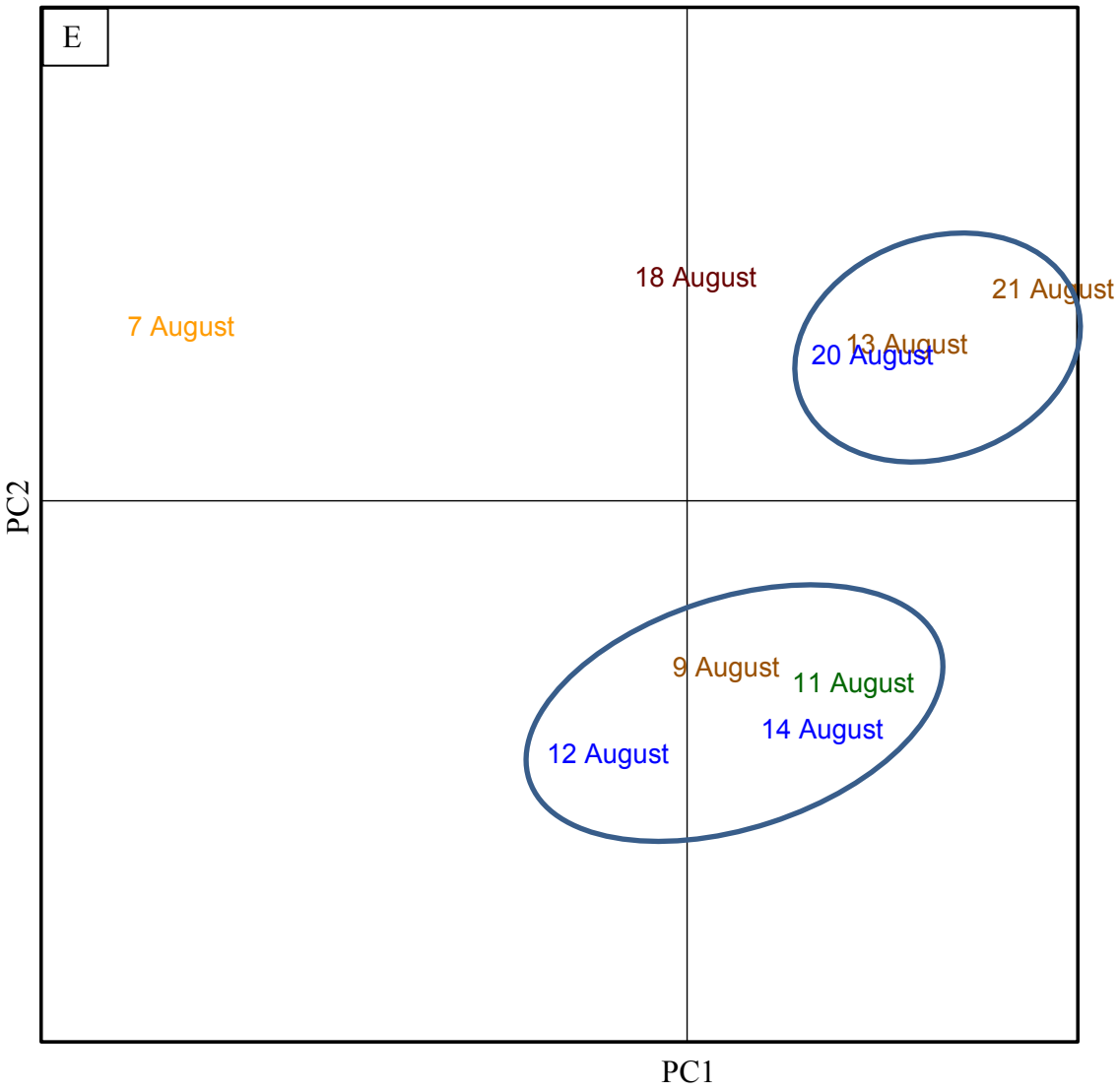


Legend:
Dead
Less than 10,001 bees
10,001-20,000 bees
20,001-30,000 bees
30,001-40,000 bees
40,001-50,000 bees
50,001-50,000 bees

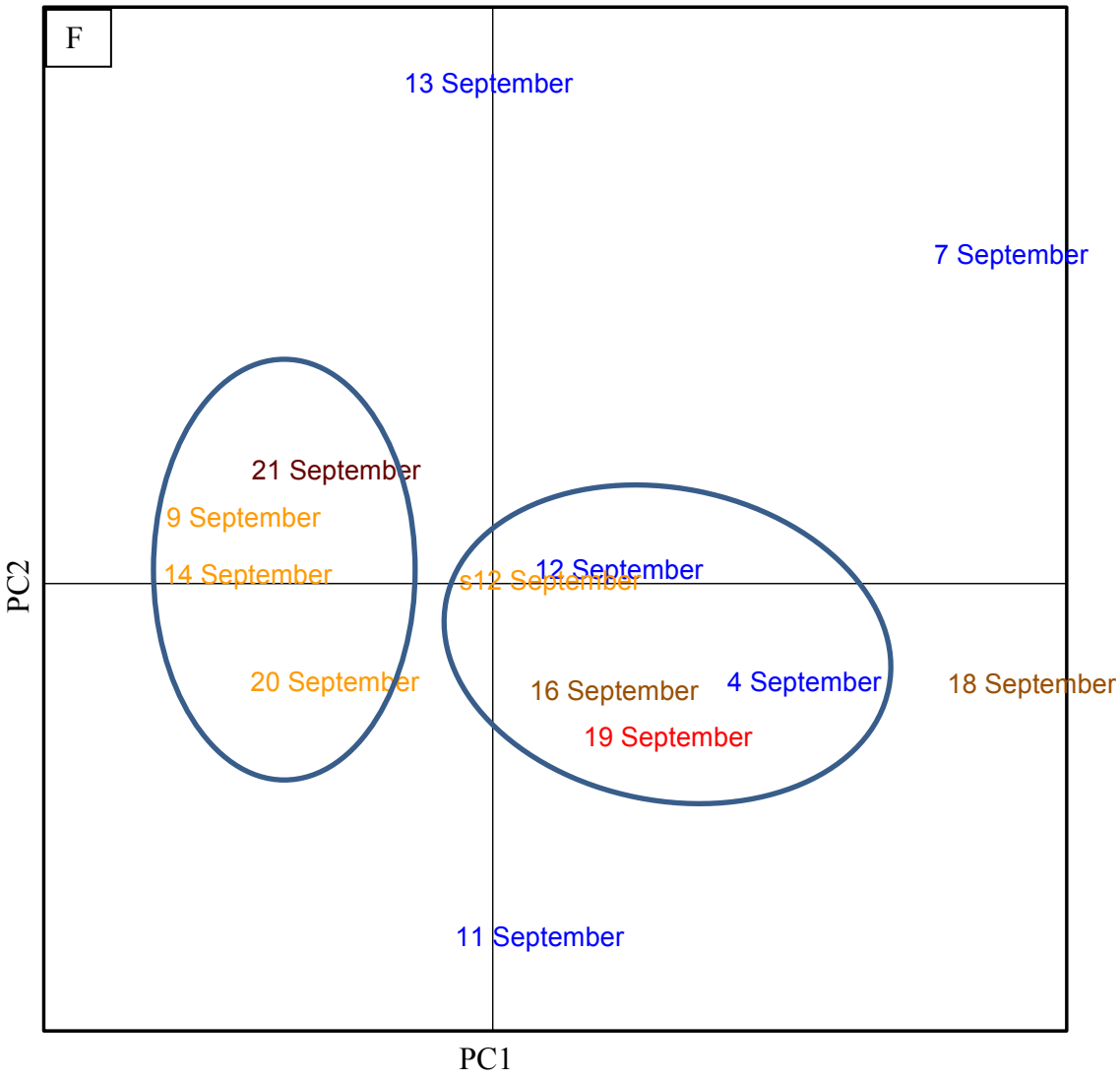


Legend:
Dead
Less than 10,001 bees
10,001-20,000 bees
20,001-30,000 bees
30,001-40,000 bees
40,001-50,000 bees
50,001-50,000 bees

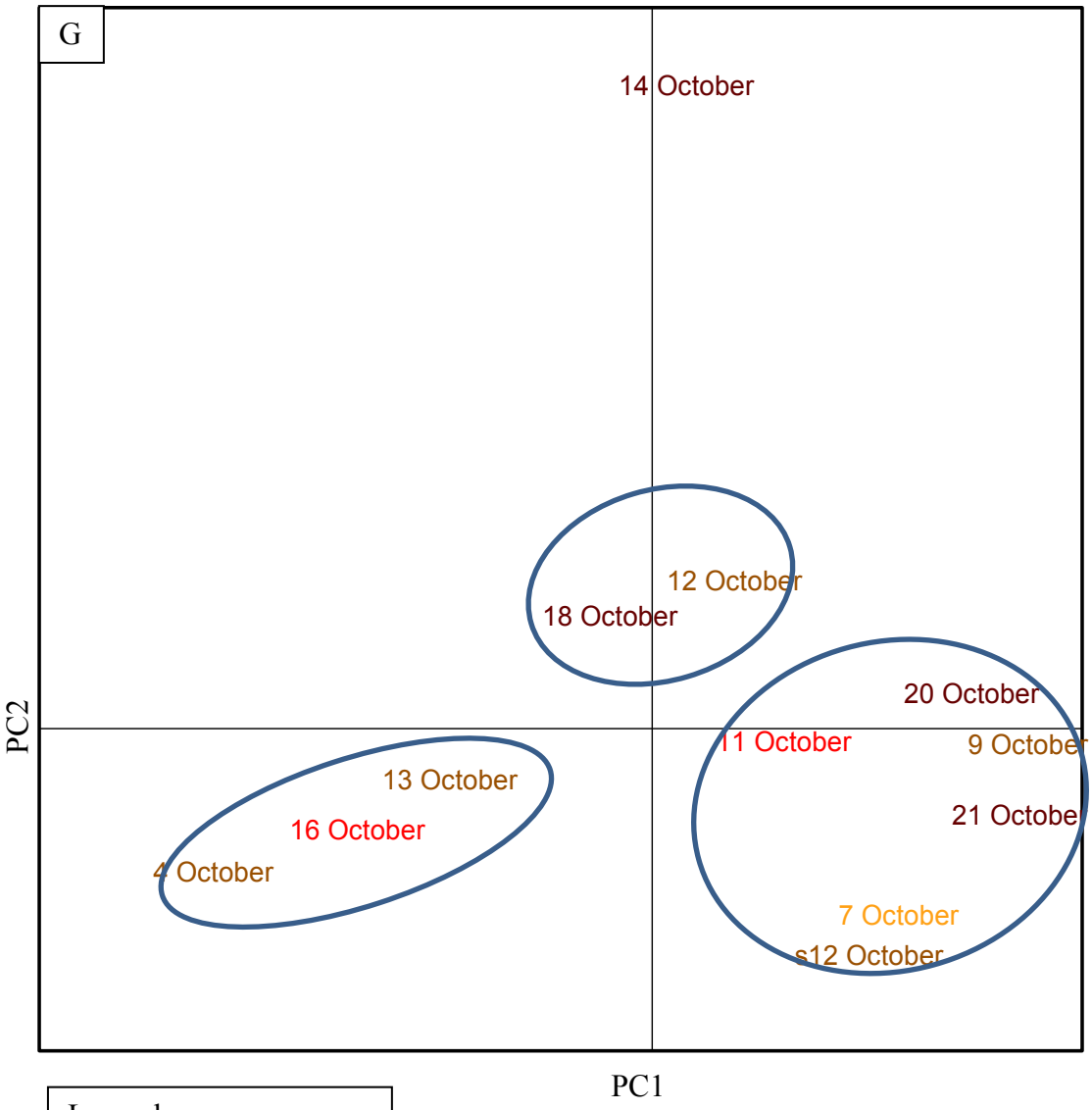

D
13 July
14 July
16 July
19 July
4 July
7 July
18 July
s3 July
s12 July
21 July
11 July
20 July
12 July
9 July
PC2
PC1
Legend:
Dead
Less than 10,001 bees
10,001-20,000 bees
20,001-30,000 bees
30,001-40,000 bees
40,001-50,000 bees
50,001-50,000 bees



Legend:
Dead
Less than 10,001 bees
10,001-20,000 bees
20,001-30,000 bees
30,001-40,000 bees
40,001-50,000 bees
50,001-50,000 bees



Legend:
Dead
Less than 10,001 bees
10,001-20,000 bees
20,001-30,000 bees
30,001-40,000 bees
40,001-50,000 bees
50,001-50,000 bees

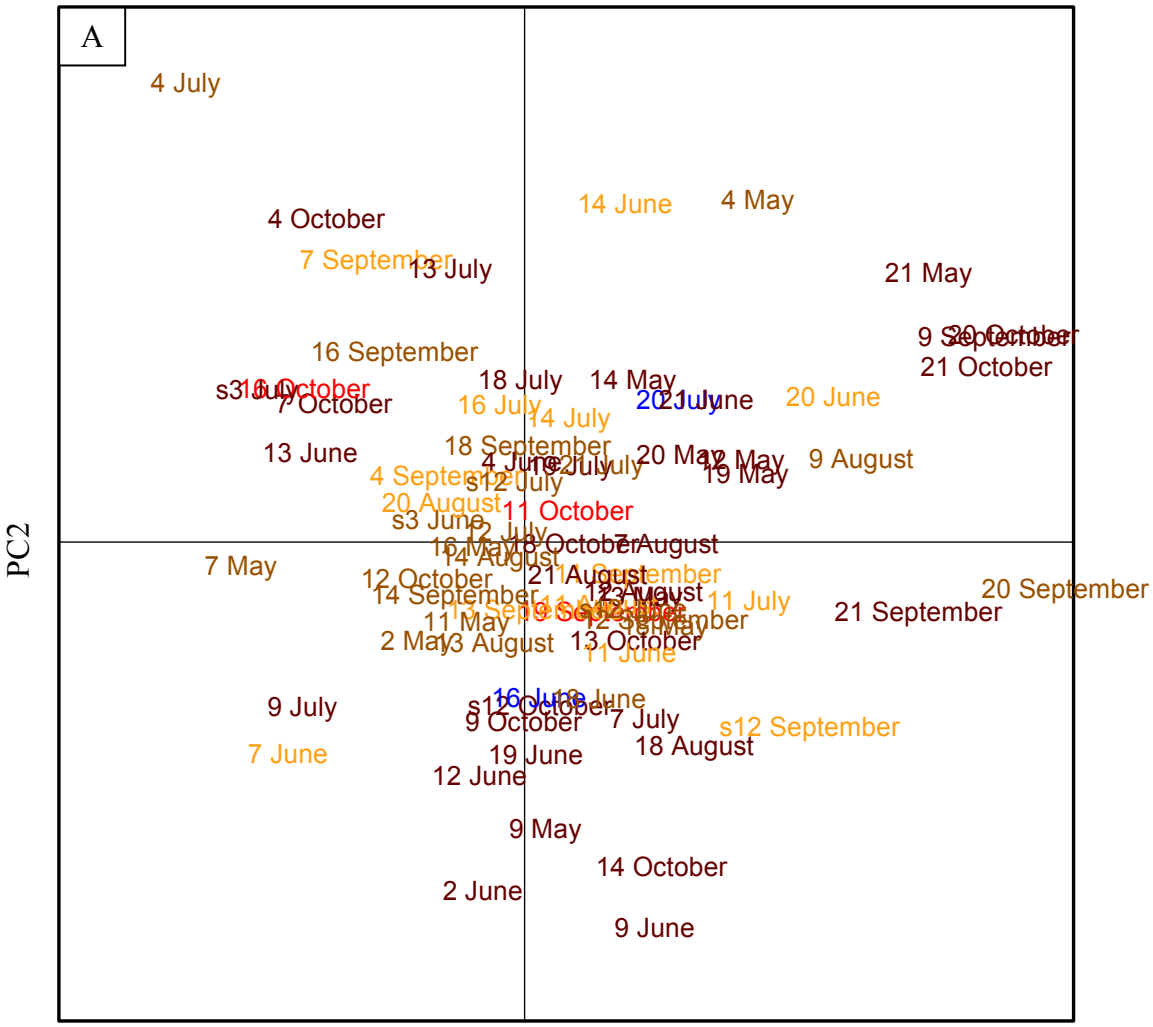


Legend:
Dead
Less than 10,001 bees
10,001-20,000 bees
20,001-30,000 bees
30,001-40,000 bees
40,001-50,000 bees
50,001-50,000 bees

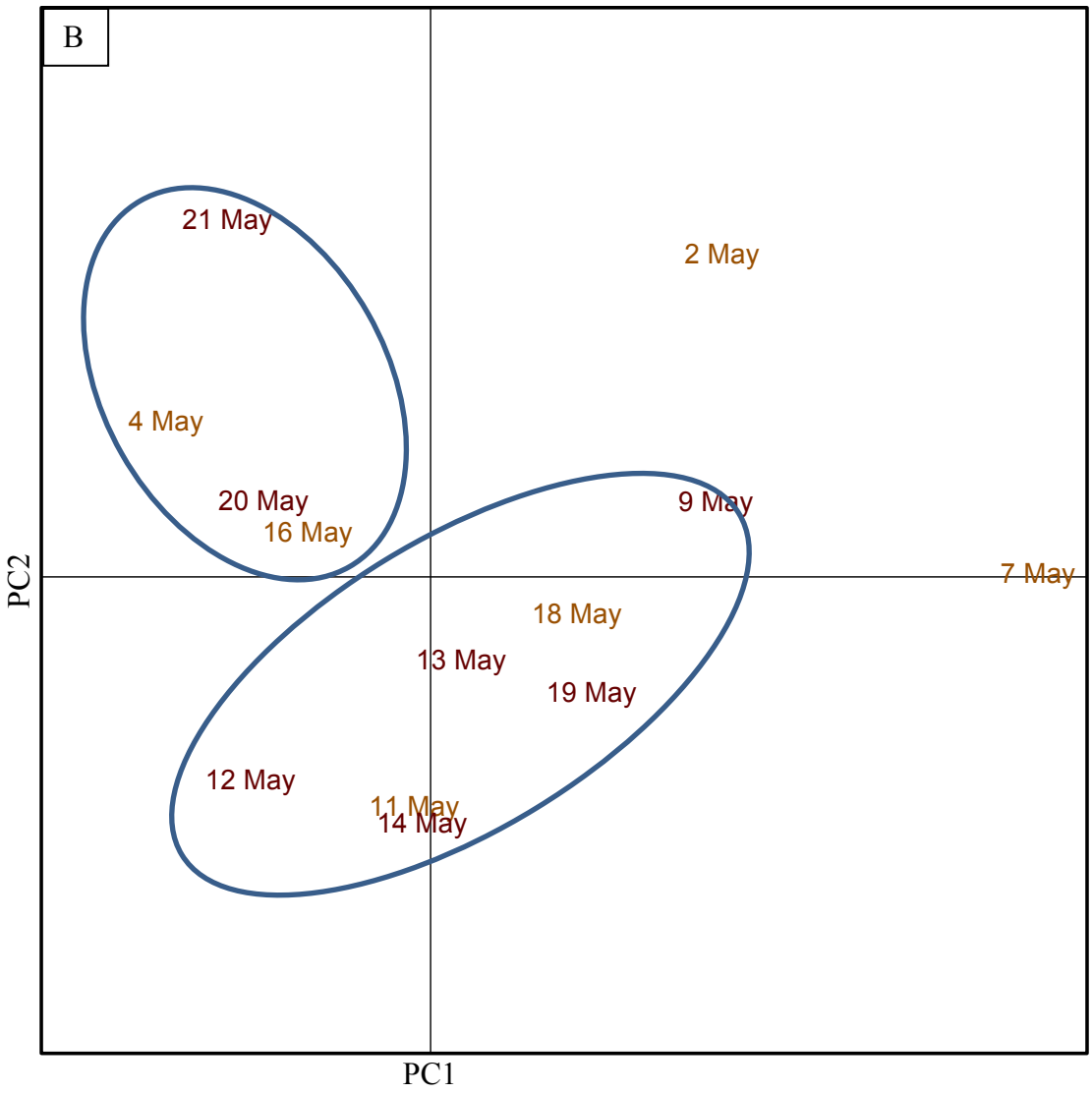
Figure B1 A-G: PCA of all DGGE bacterial profiles of bee bread sampled monthly from the South Campus Research Apiary by the amount of bees. Preceding numbers indicate which colony the bee bread was sampled from and the following month indicates the month the bee bread was sampled. A.) 2D PCA of all bee bread samples. The first and second components represent 5.4% and 5.0% of the variation respectively. B.) 2D PCA of May bee bread samples. The first and second components represent 16.8% and 12.8% of the variation respectively. C.) 2D PCA of June bee bread samples. The first and second components represent 15.2% and 11.3% of the variation respectively. D.) 2D PCA of July bee bread samples. The first and second components represent 14.9% and 12.8% of the variation respectively. E.) 2D PCA of August bee bread samples. The first and second components represent 19.4% and 16.5% of the variation respectively. F.) 2D PCA of September bee bread samples. The first and and second components represents 14.6% and 12.2% of the variation respectively. G.) 2D PCA of October bee bread samples. The first and second components represent 17.3% and 14.9% of the variation respectively.

APPENDIX C

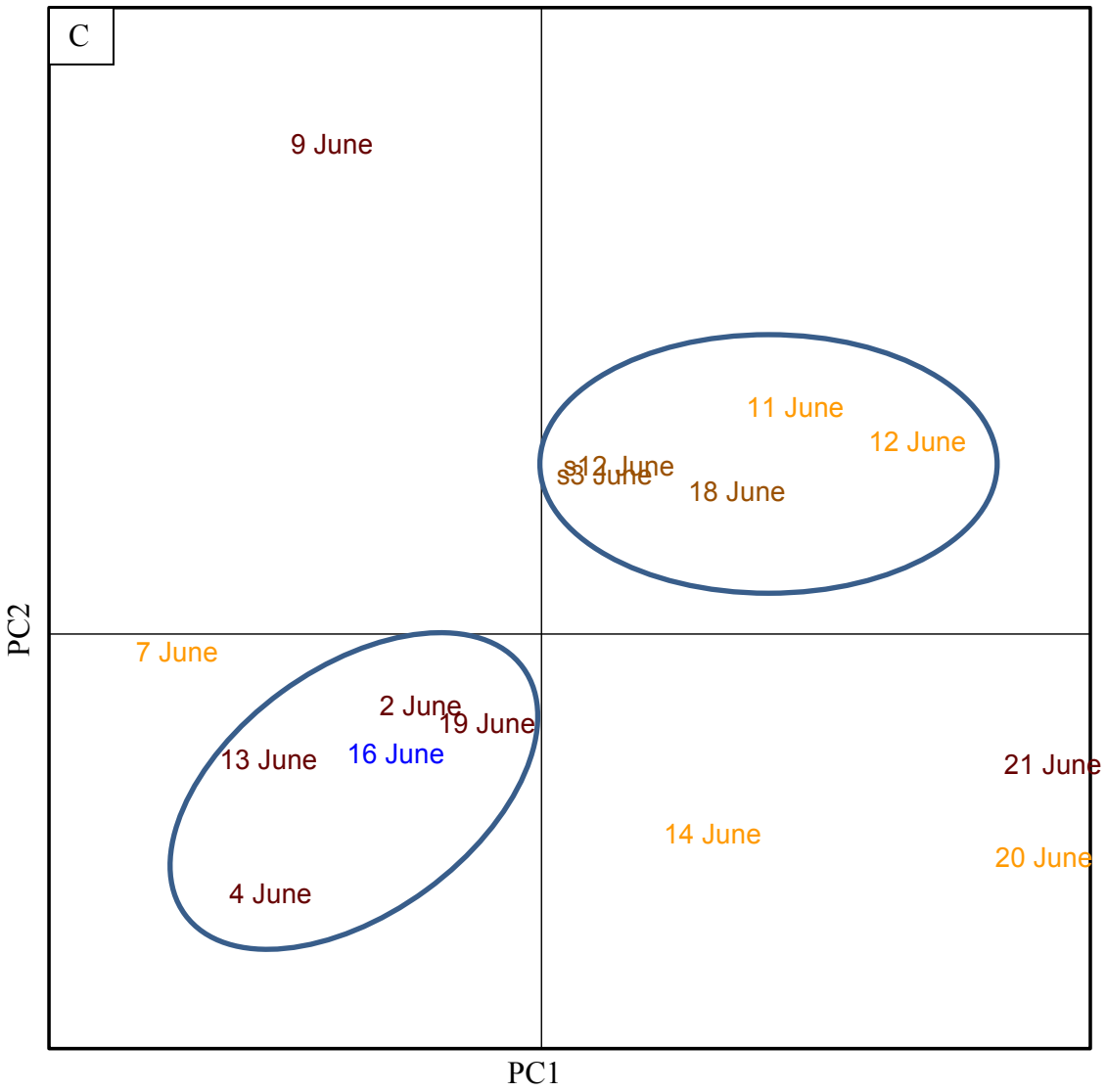
PCA OF ALL BEE BREAD BACTERIAL PROFILES BY THE NUMBER OF BROOD CELLS



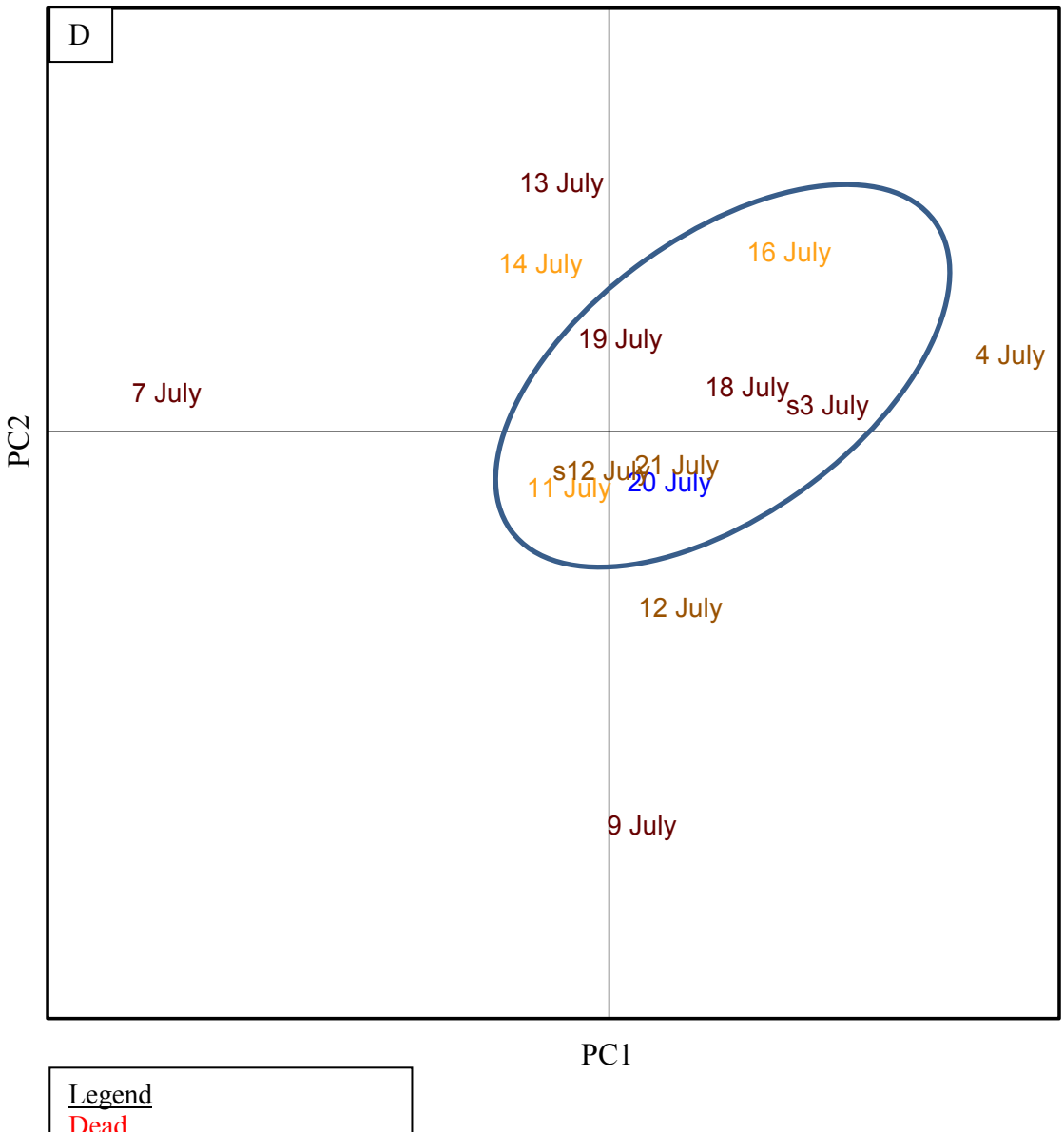
Legend
Dead
Less than 10,000 brood cells
10,001-20,000 brood cells
20,001-30,000 brood cells
30,001-40,000 brood cells



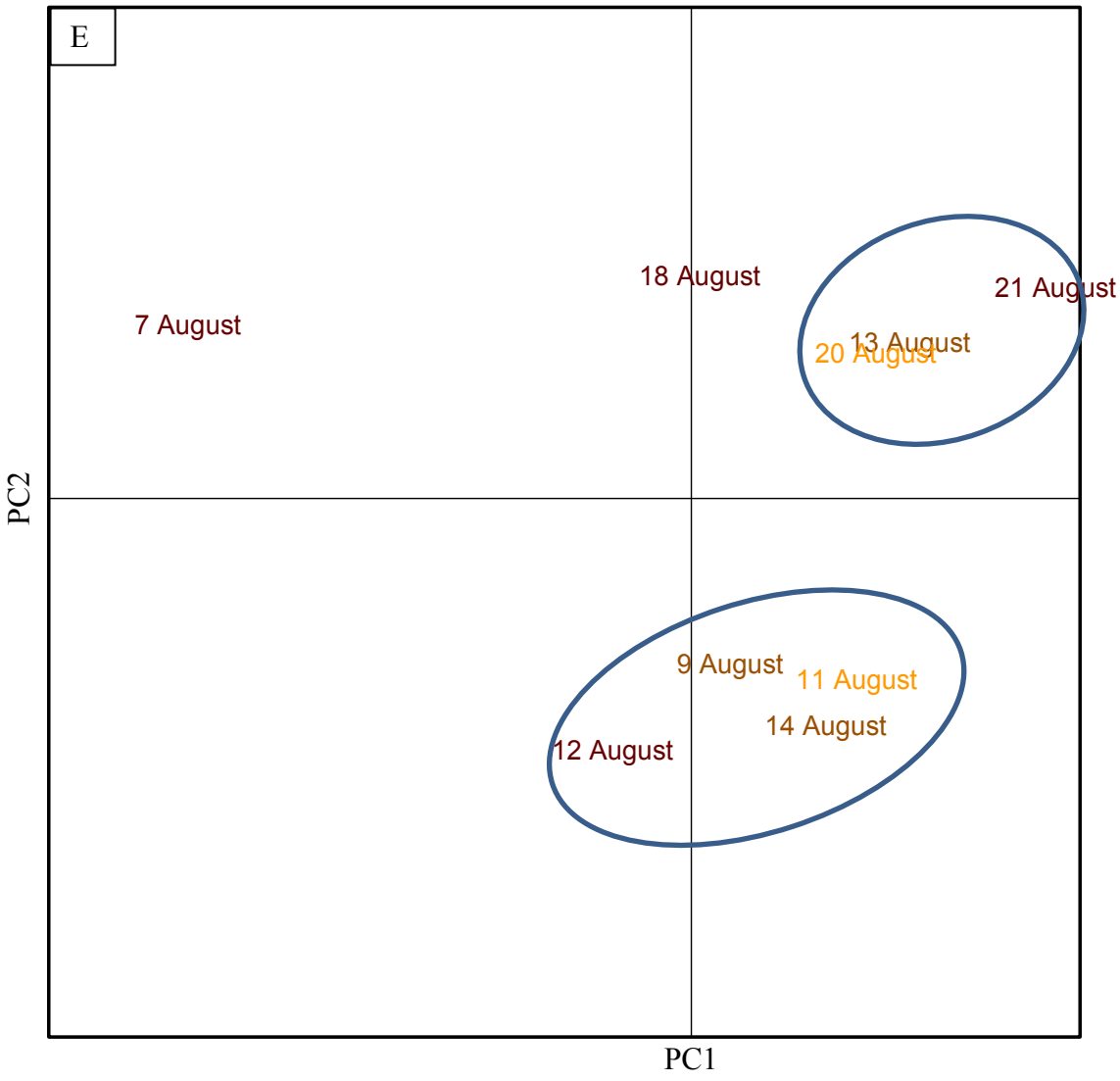
Legend
Dead
Less than 10,000 brood cells
10,001-20,000 brood cells
20,001-30,000 brood cells
30,001-40,000 brood cells



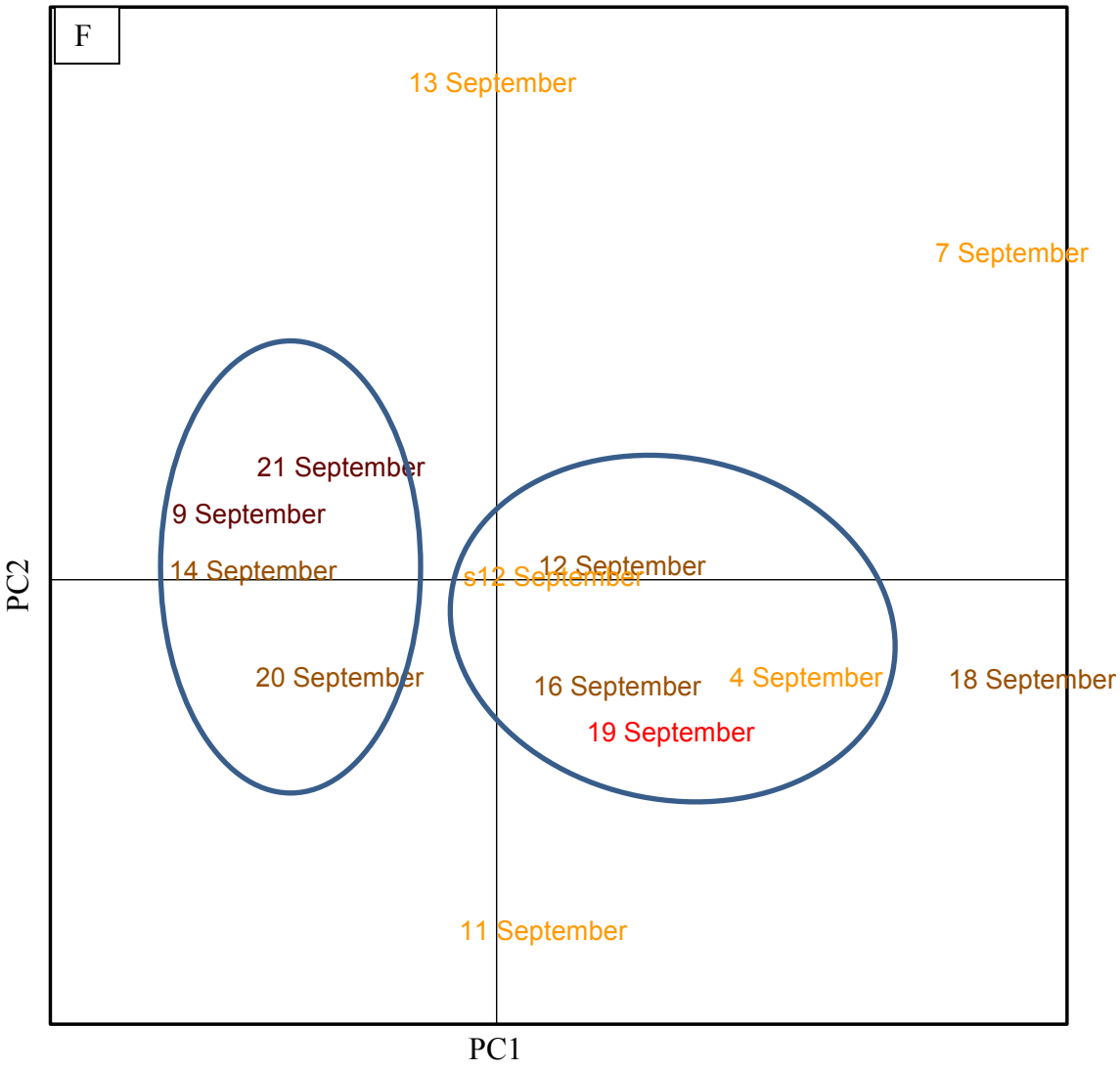
Legend
Dead
Less than 10,000 brood cells
10,001-20,000 brood cells
20,001-30,000 brood cells
30,001-40,000 brood cells



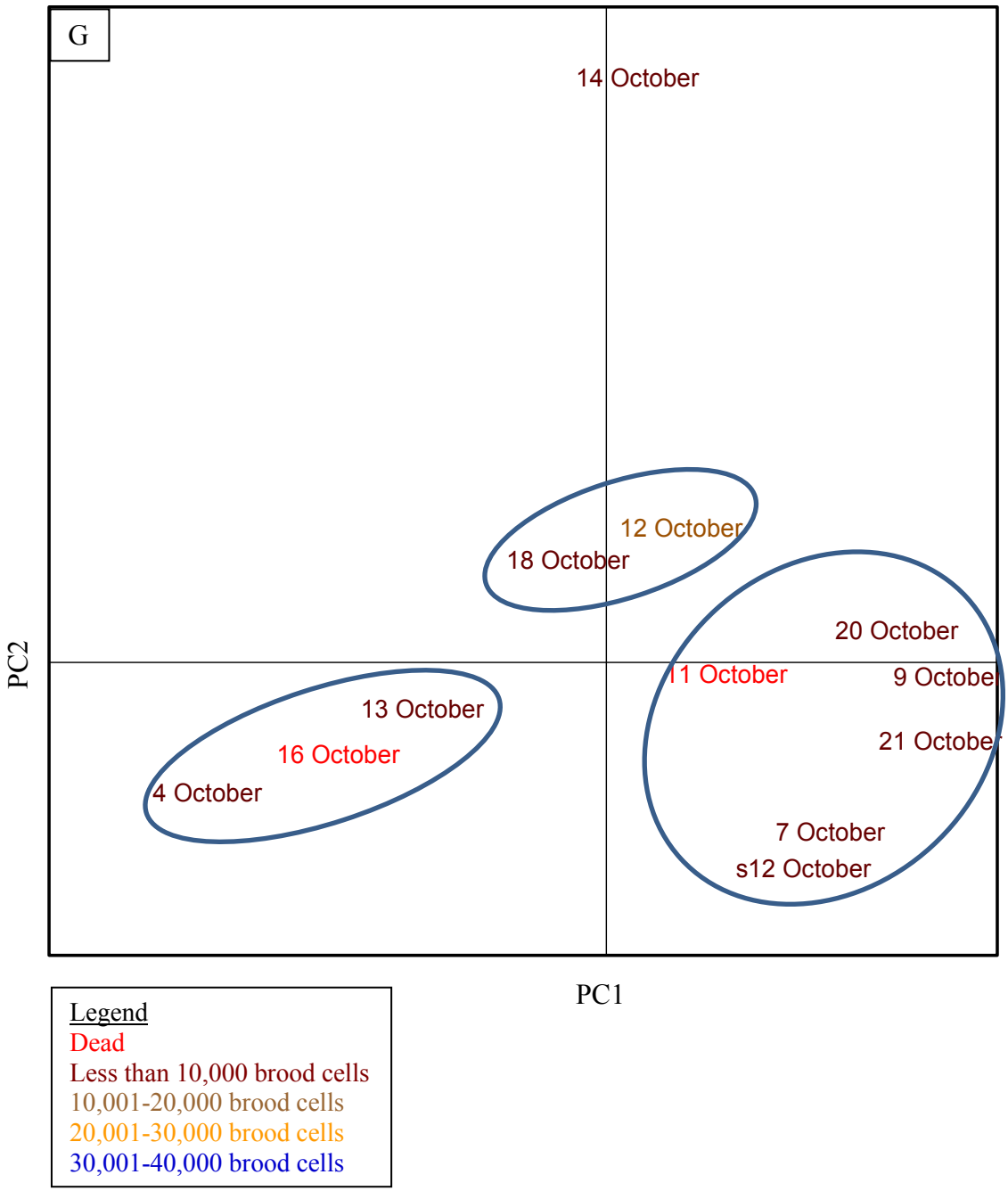
Legend
Dead
Less than 10,000 brood cells
10,001-20,000 brood cells
20,001-30,000 brood cells
30,001-40,000 brood cells



Legend
Dead
Less than 10,000 brood cells
10,001-20,000 brood cells
20,001-30,000 brood cells
30,001-40,000 brood cells



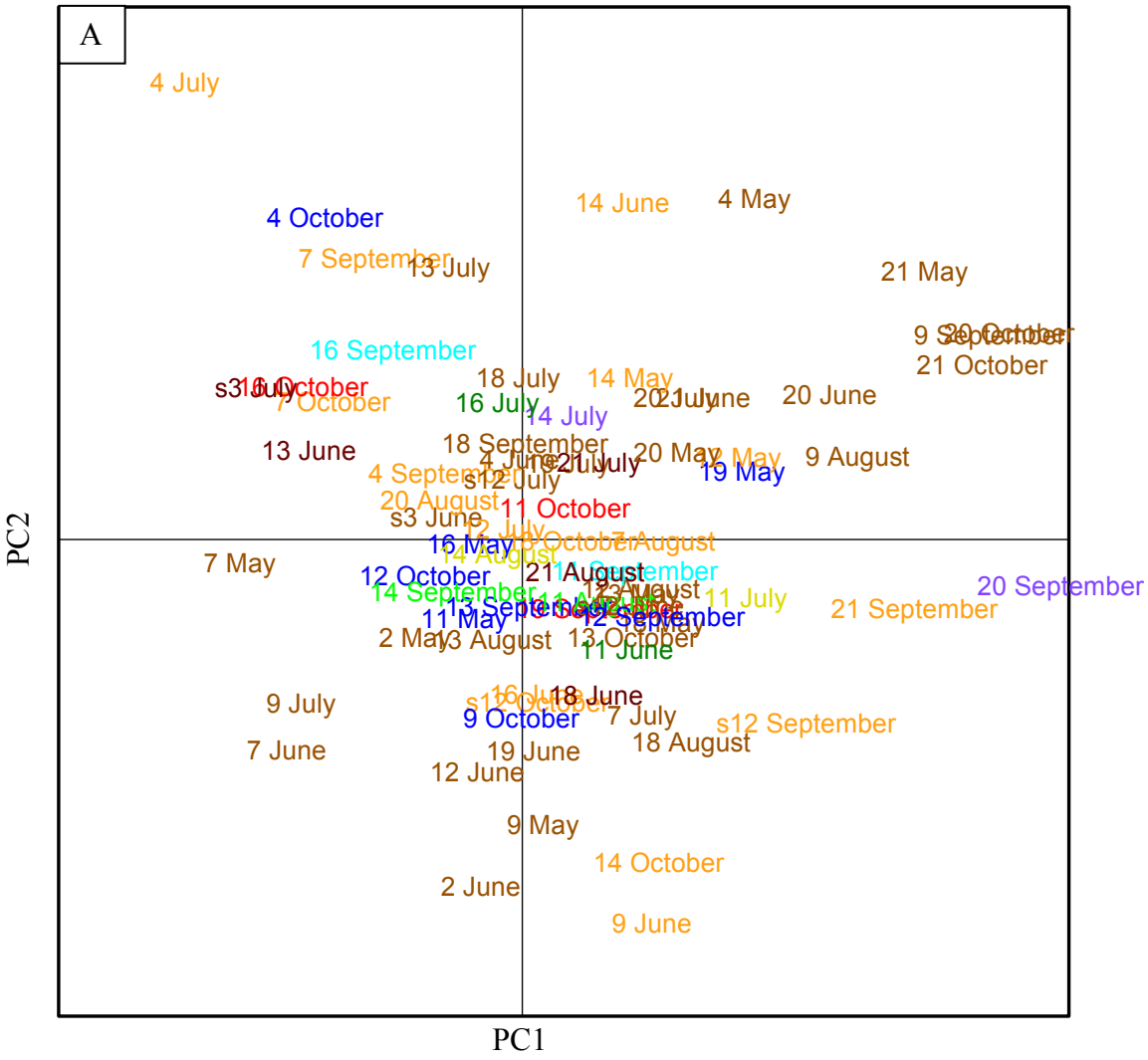
Legend
Dead
Less than 10,000 brood cells
10,001-20,000 brood cells
20,001-30,000 brood cells
30,001-40,000 brood cells


G
14 October
12 October
18 October
20 October
11 October
9 October
21 October
13 October
16 October
4 October
7 October
s12 October
PC2
PC1
Legend
Dead
Less than 10,000 brood cells
10,001-20,000 brood cells
20,001-30,000 brood cells
30,001-40,000 brood cells

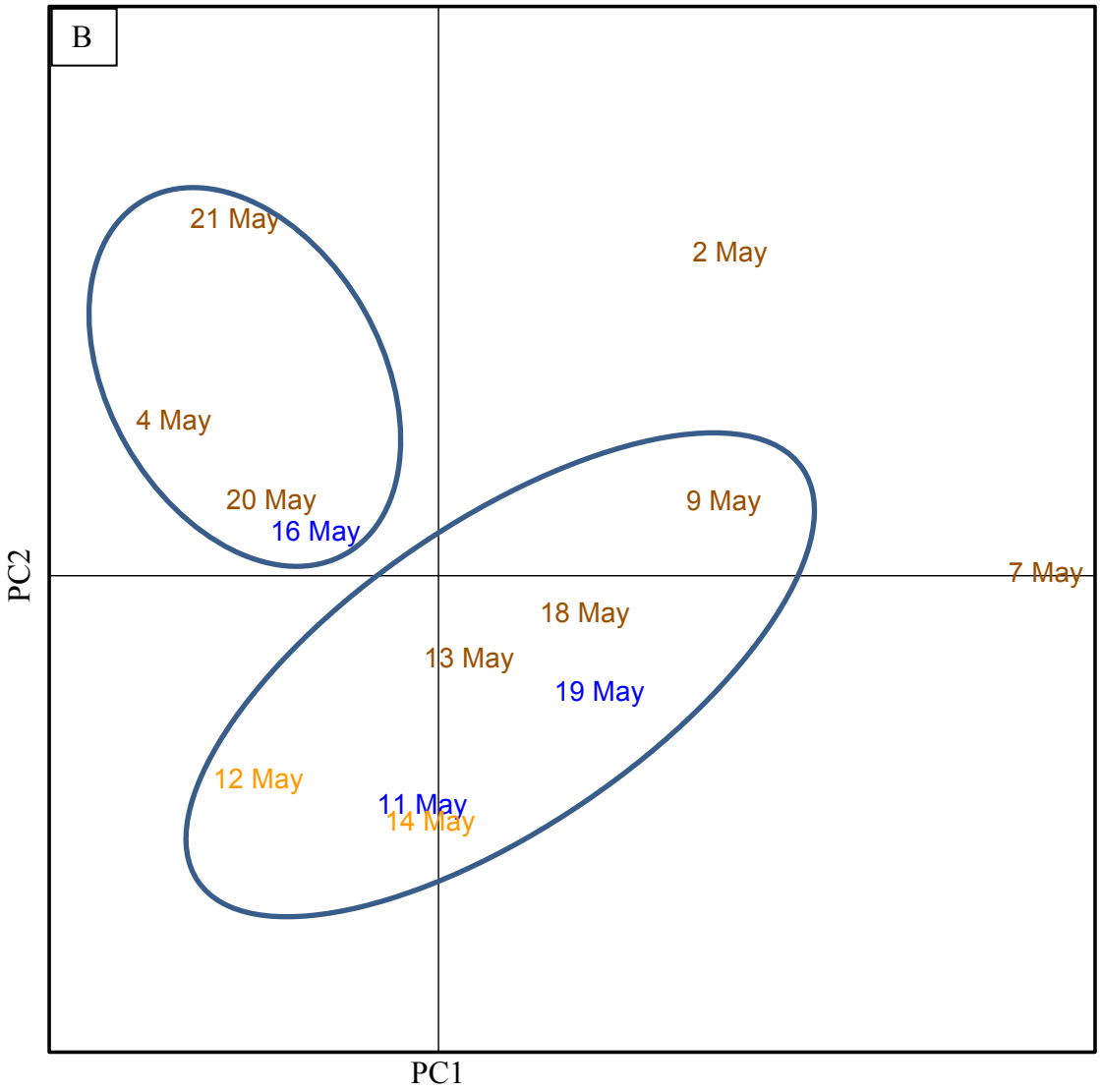
Figures C1 A-G: PCA of all DGGE bacterial profiles of bee bread sampled monthly from the South Campus Research Apiary by the amount of brood cells. Preceding numbers indicate which colony the bee bread was sampled from and the following month indicates the month the bee bread was sampled. A.) 2D PCA of all bee bread samples. The first and second components represent 5.4% and 5.0% of the variation respectively. B.) 2D PCA of May bee bread samples. The first and second components represent 16.8% and 12.8% of the variation respectively. C.) 2D PCA of June bee bread samples. The first and second components represent 15.2% and 11.3% of the variation respectively. D.) 2D PCA of July bee bread samples. The first and second components represent 14.9% and 12.8% of the variation respectively. E.) 2D PCA of August bee bread samples. The first and second components represent 19.4% and 16.5% of the variation respectively. F.) 2D PCA of September bee bread samples. The first and and second components represents 14.6% and 12.2% of the variation respectively. G.) 2D PCA of October bee bread samples. The first and second components represent 17.3% and 14.9% of the variation respectively.

APPENDIX D

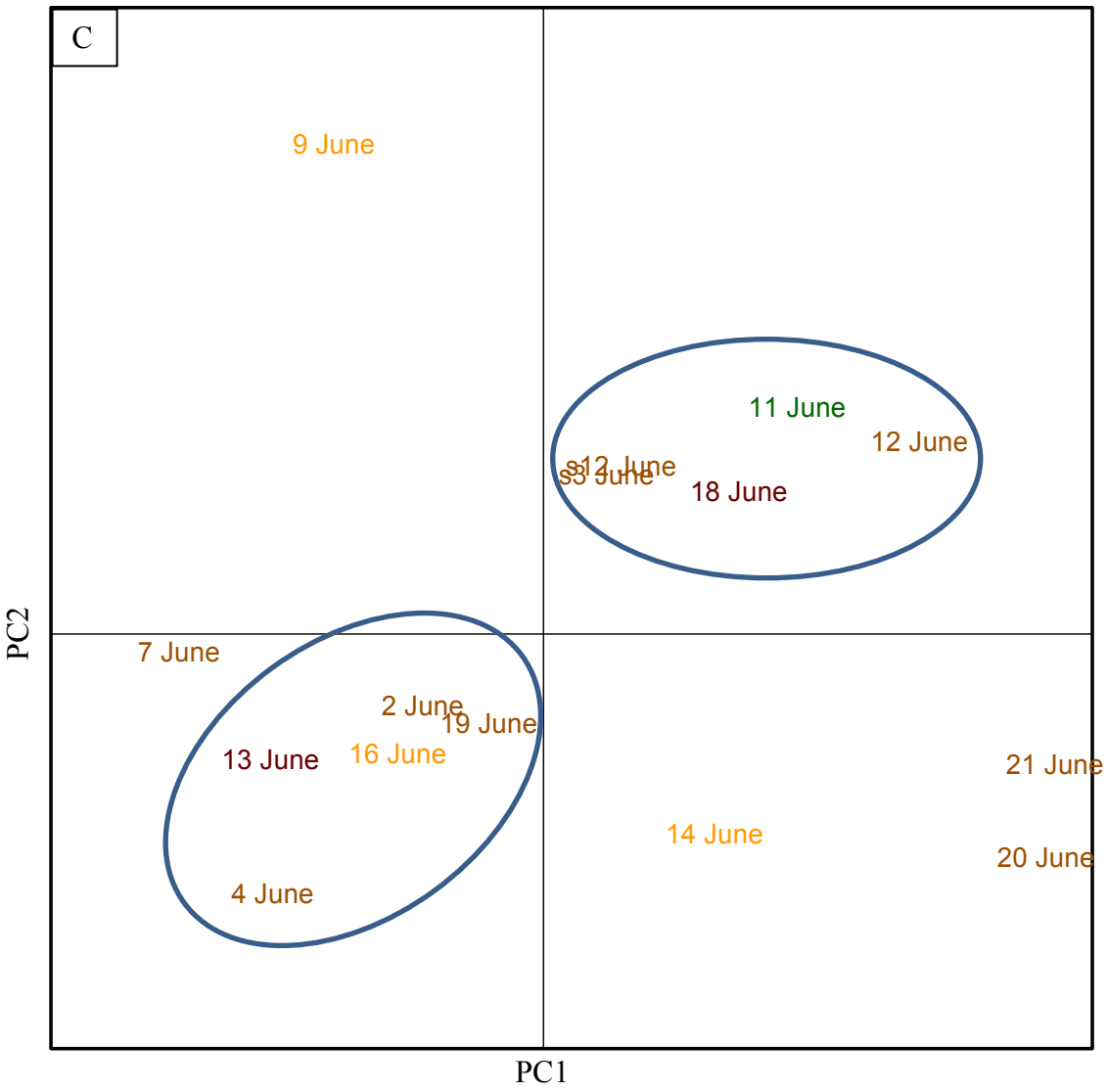
PCA OF ALL BEE BREAD BACTERIAL PROFILES BY THE AMOUNT OF MITES



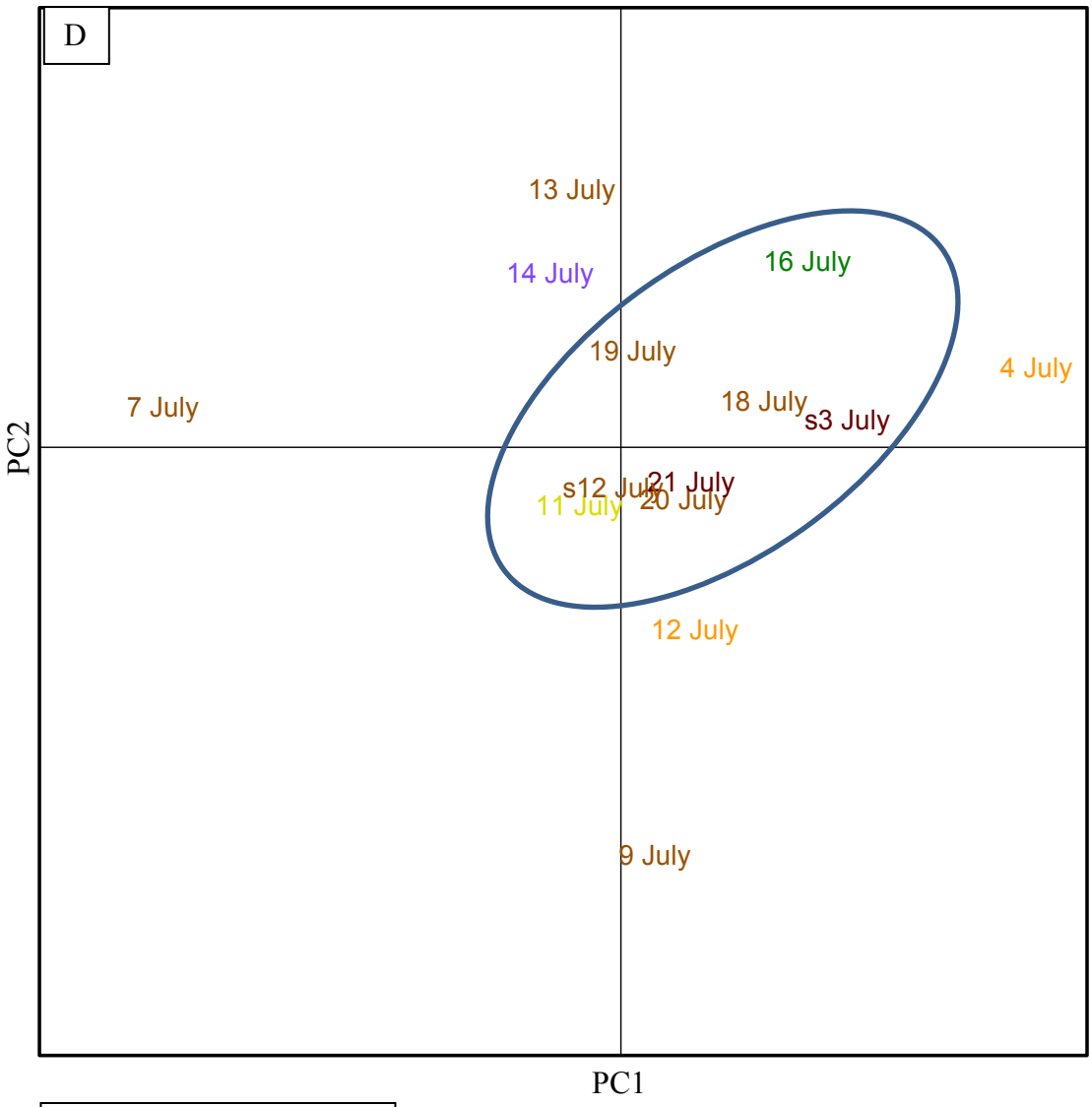
Legend
Dead
0 mites
1-10 mites
11-20 mites
21-30 mites
31-50 mites
51-70 mites
71-100 mites
101-200 mites
201-300 mites



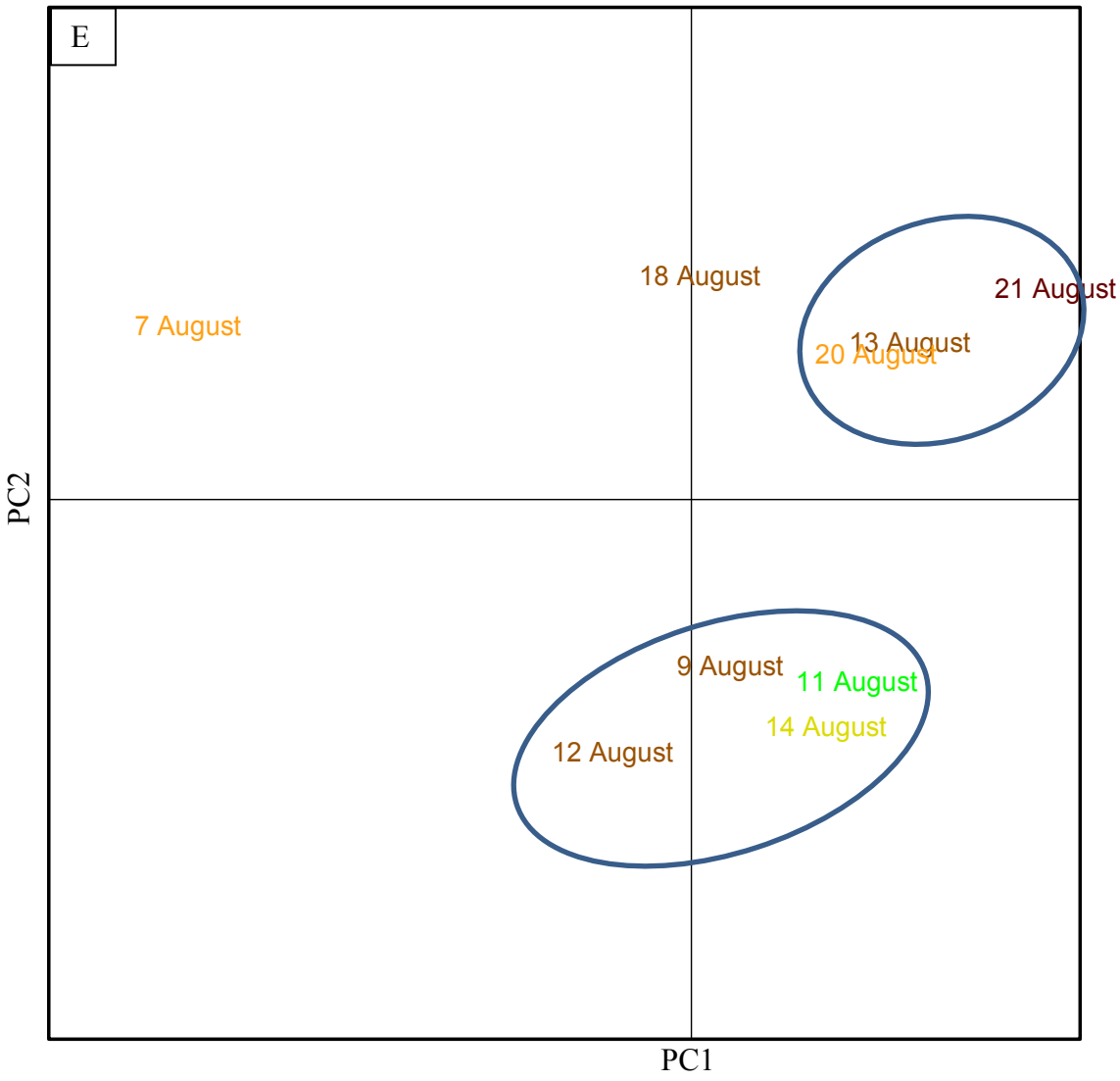
Legend
Dead
0 mites
1-10 mites
11-20 mites
21-30 mites
31-50 mites
51-70 mites
71-100 mites
101-200 mites
201-300 mites



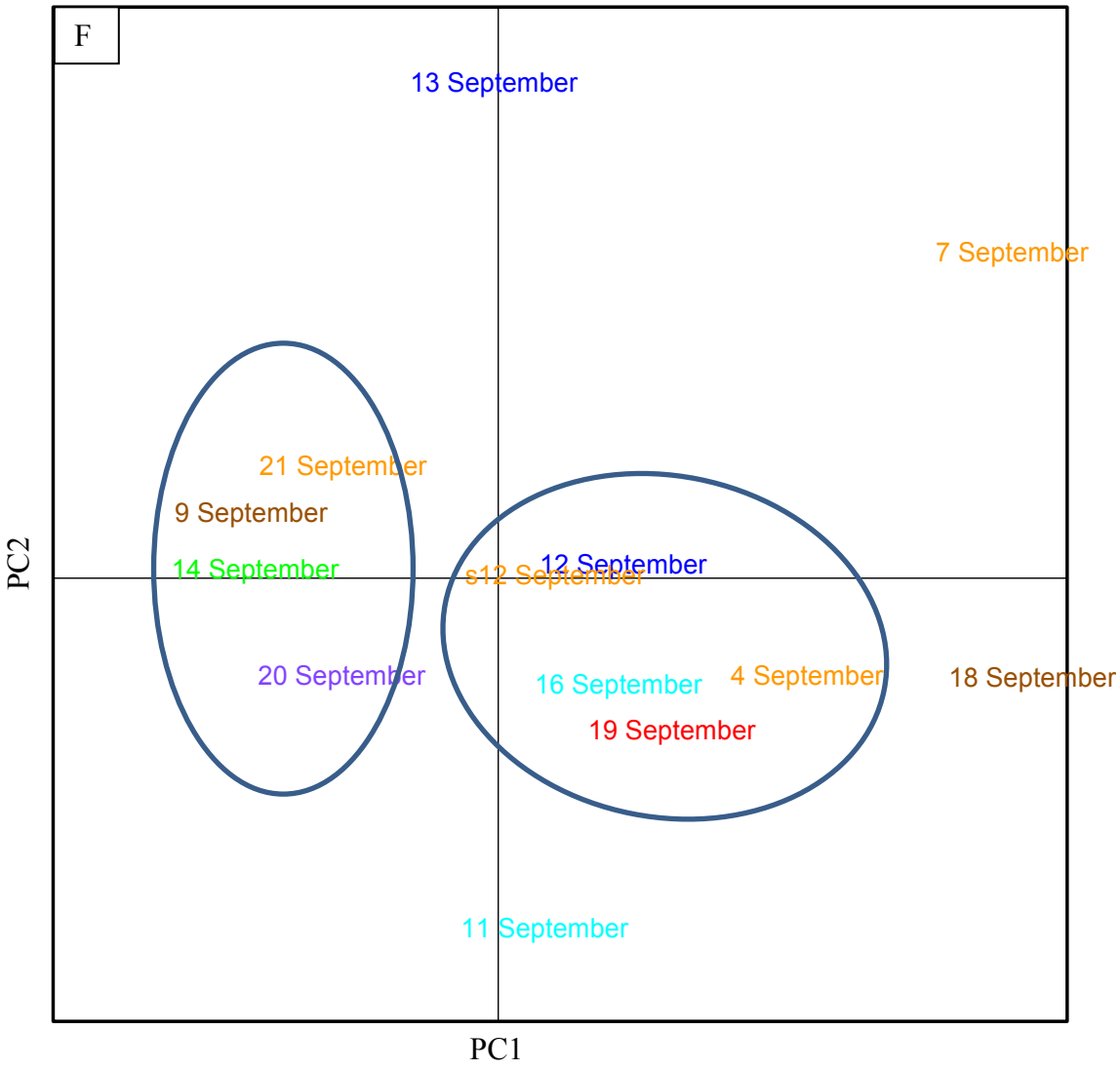
Legend
Dead
0 mites
1-10 mites
11-20 mites
21-30 mites
31-50 mites
51-70 mites
71-100 mites
101-200 mites
201-300 mites



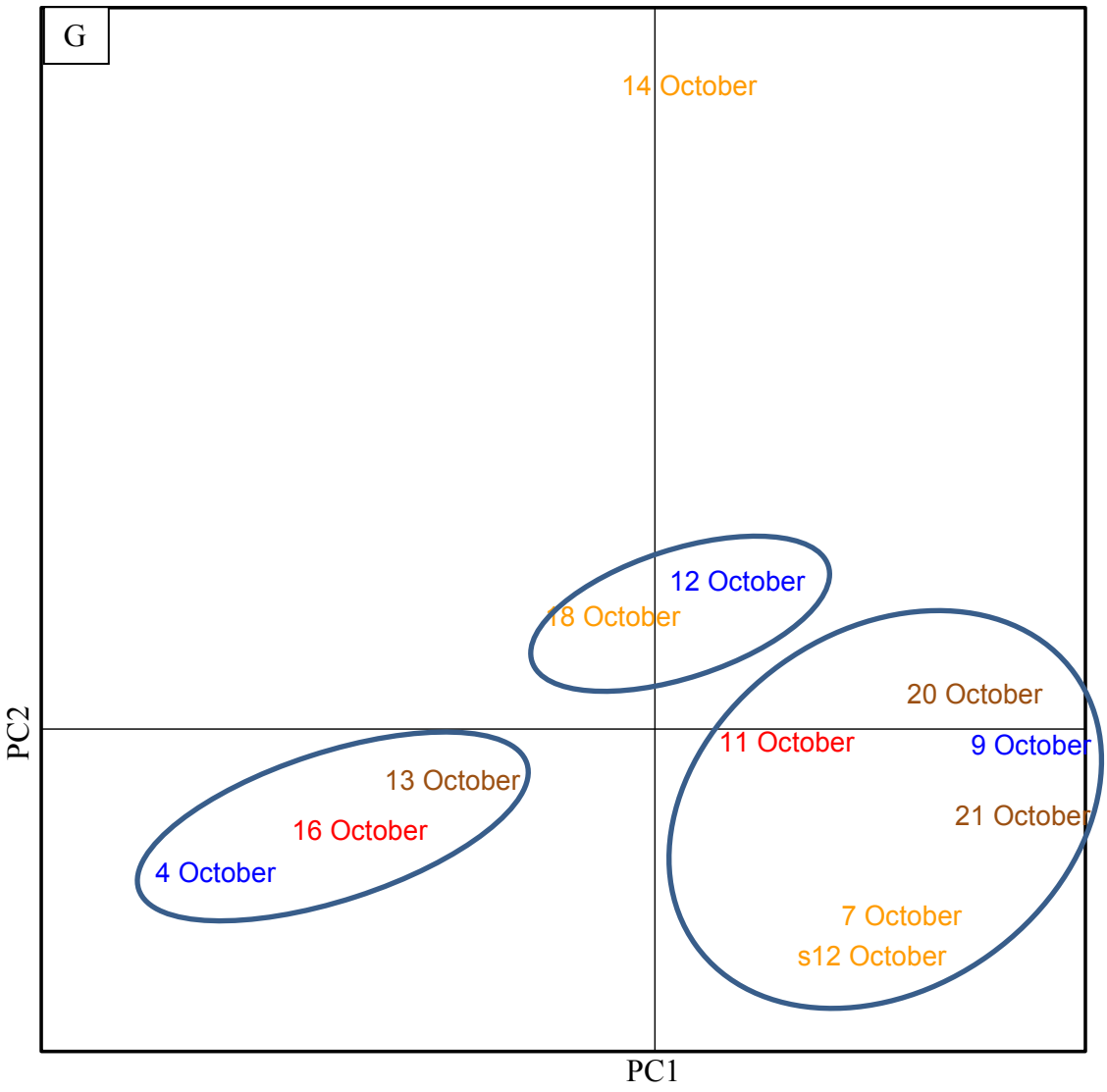
Legend
Dead
0 mites
1-10 mites
11-20 mites
21-30 mites
31-50 mites
51-70 mites
71-100 mites
101-200 mites
201-300 mites



Legend
Dead
0 mites
1-10 mites
11-20 mites
21-30 mites
31-50 mites
51-70 mites
71-100 mites
101-200 mites
201-300 mites



Legend
Dead
0 mites
1-10 mites
11-20 mites
21-30 mites
31-50 mites
51-70 mites
71-100 mites
101-200 mites
201-300 mites

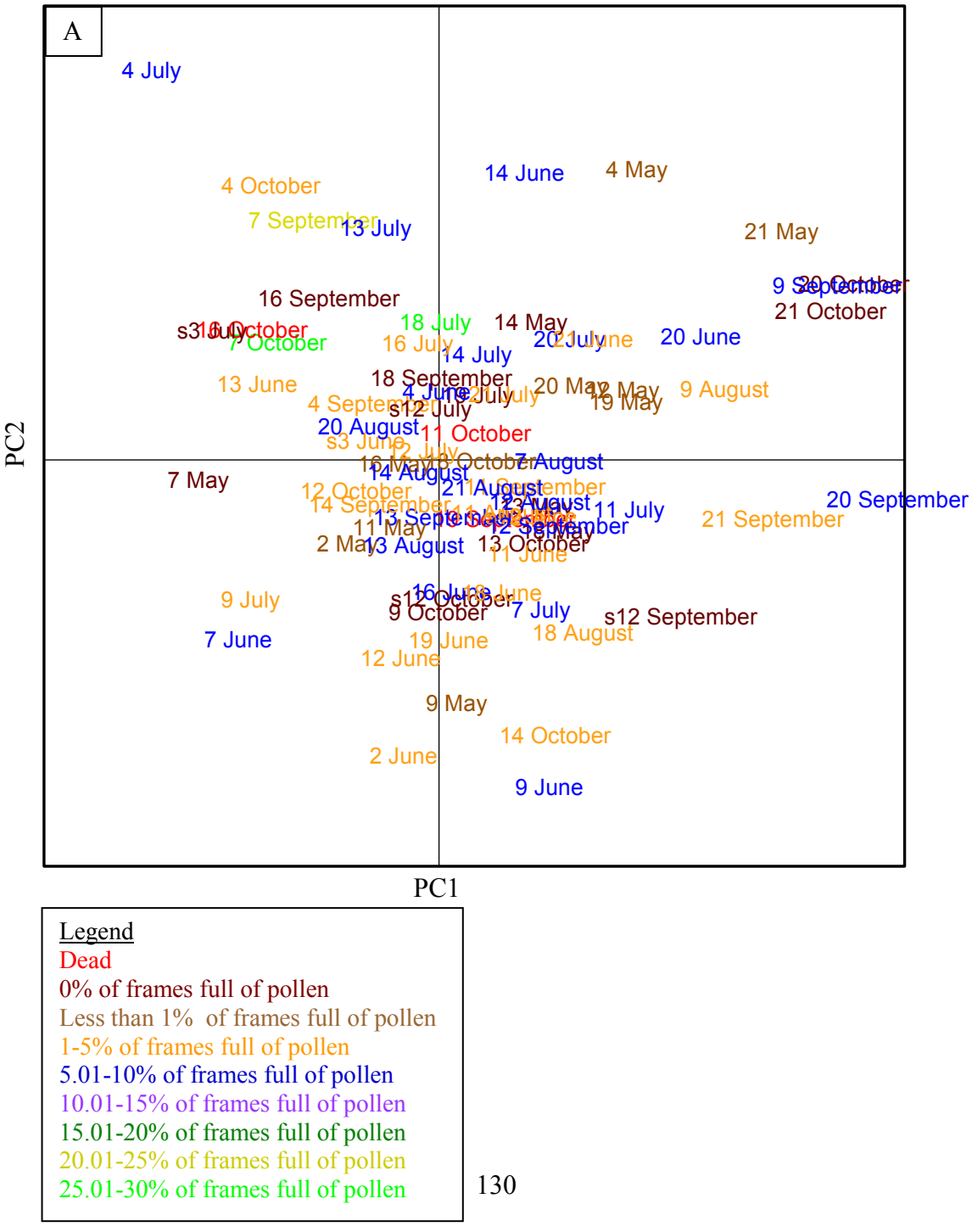


Legend
Dead
0 mites
1-10 mites
11-20 mites
21-30 mites
31-50 mites
51-70 mites
71-100 mites
101-200 mites
201-300 mites

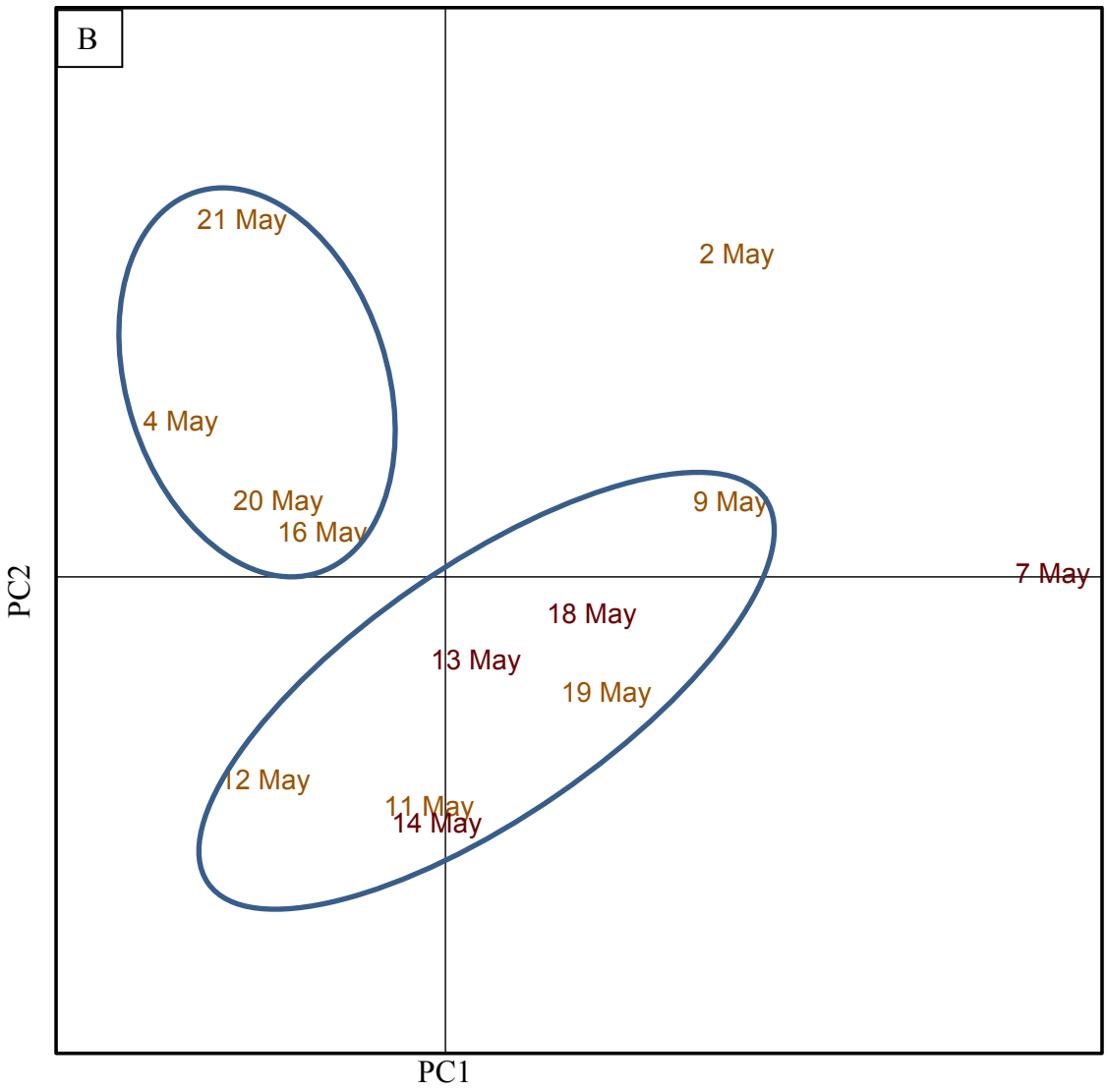
Figure D1 A-G: PCA of all DGGE bacterial profiles of bee bread sampled monthly from the South Campus Research Apiary by the amount of mites. Preceding numbers indicate which colony the bee bread was sampled from and the following month indicates the month the bee bread was sampled. A.) 2D PCA of all bee bread samples. The first and second components represent 5.4% and 5.0% of the variation respectively. B.) 2D PCA of May bee bread samples. The first and second components represent 16.8% and 12.8% of the variation respectively. C.) 2D PCA of June bee bread samples. The first and second components represent 15.2% and 11.3% of the variation respectively. D.) 2D PCA of July bee bread samples. The first and second components represent 14.9% and 12.8% of the variation respectively. E.) 2D PCA of August bee bread samples. The first and second components represent 19.4% and 16.5% of the variation respectively. F.) 2D PCA of September bee bread samples. The first and and second components represents 14.6% and 12.2% of the variation respectively. G.) 2D PCA of October bee bread samples. The first and second components represent 17.3% and 14.9% of the variation respectively.

APPENDIX E

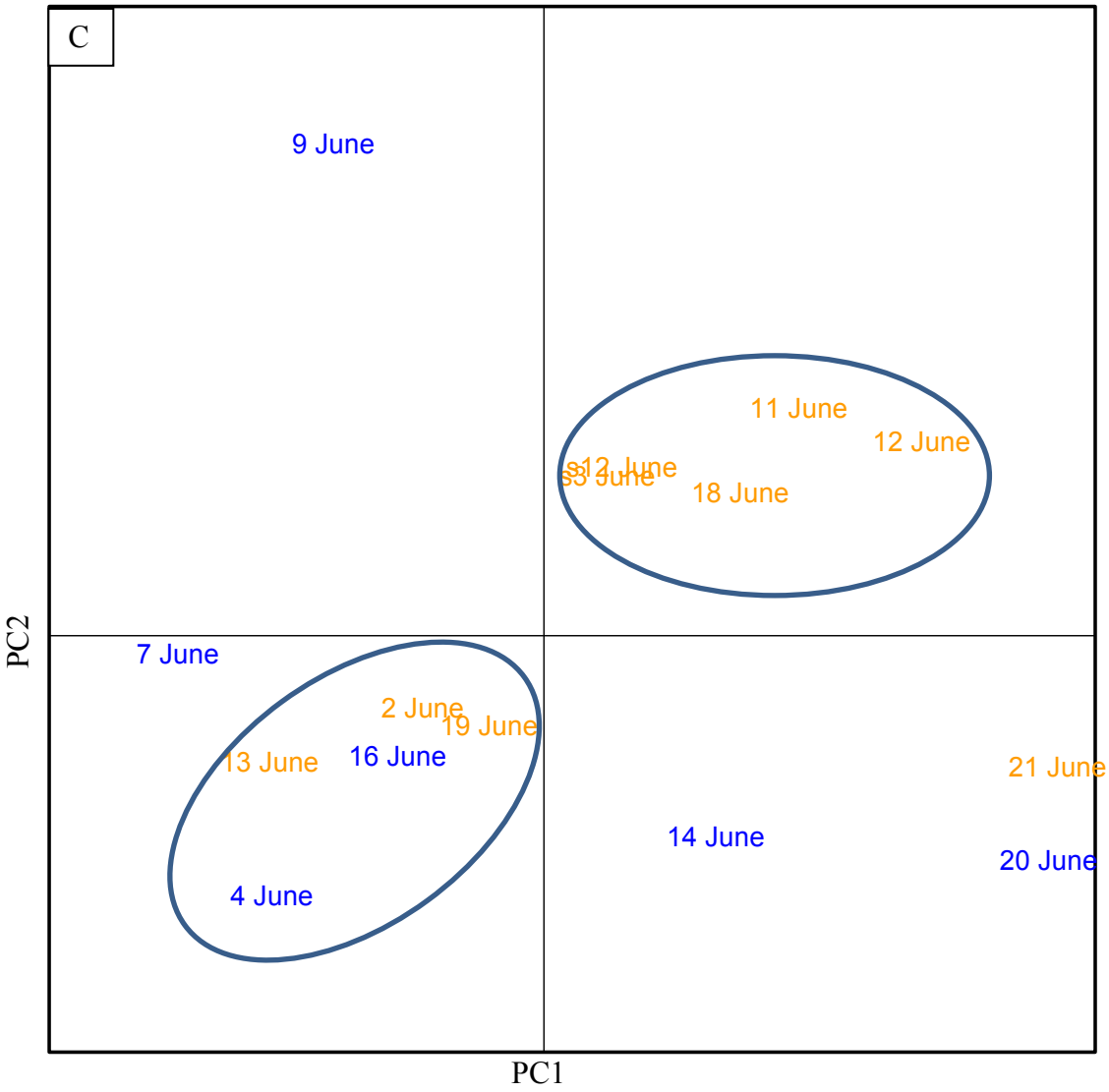
PCA OF ALL BEE BREAD BACTERIAL PROFILES BY THE AMOUNT OF POLLEN



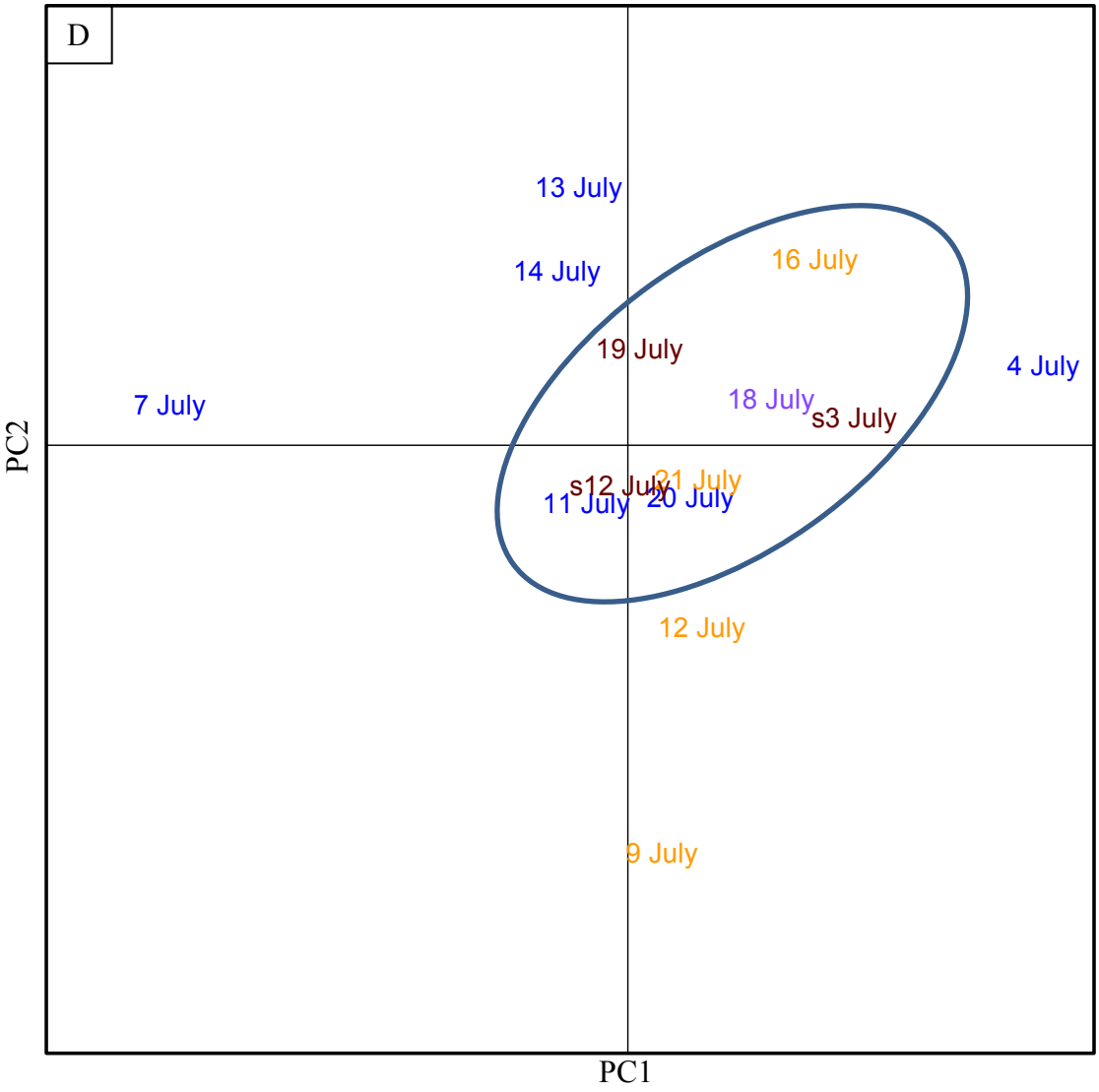
Legend
Dead
0% of frames full of pollen
Less than 1% of frames full of pollen
1-5% of frames full of pollen
5.01-10% of frames full of pollen
10.01-15% of frames full of pollen
15.01-20% of frames full of pollen
20.01-25% of frames full of pollen
25.01-30% of frames full of pollen



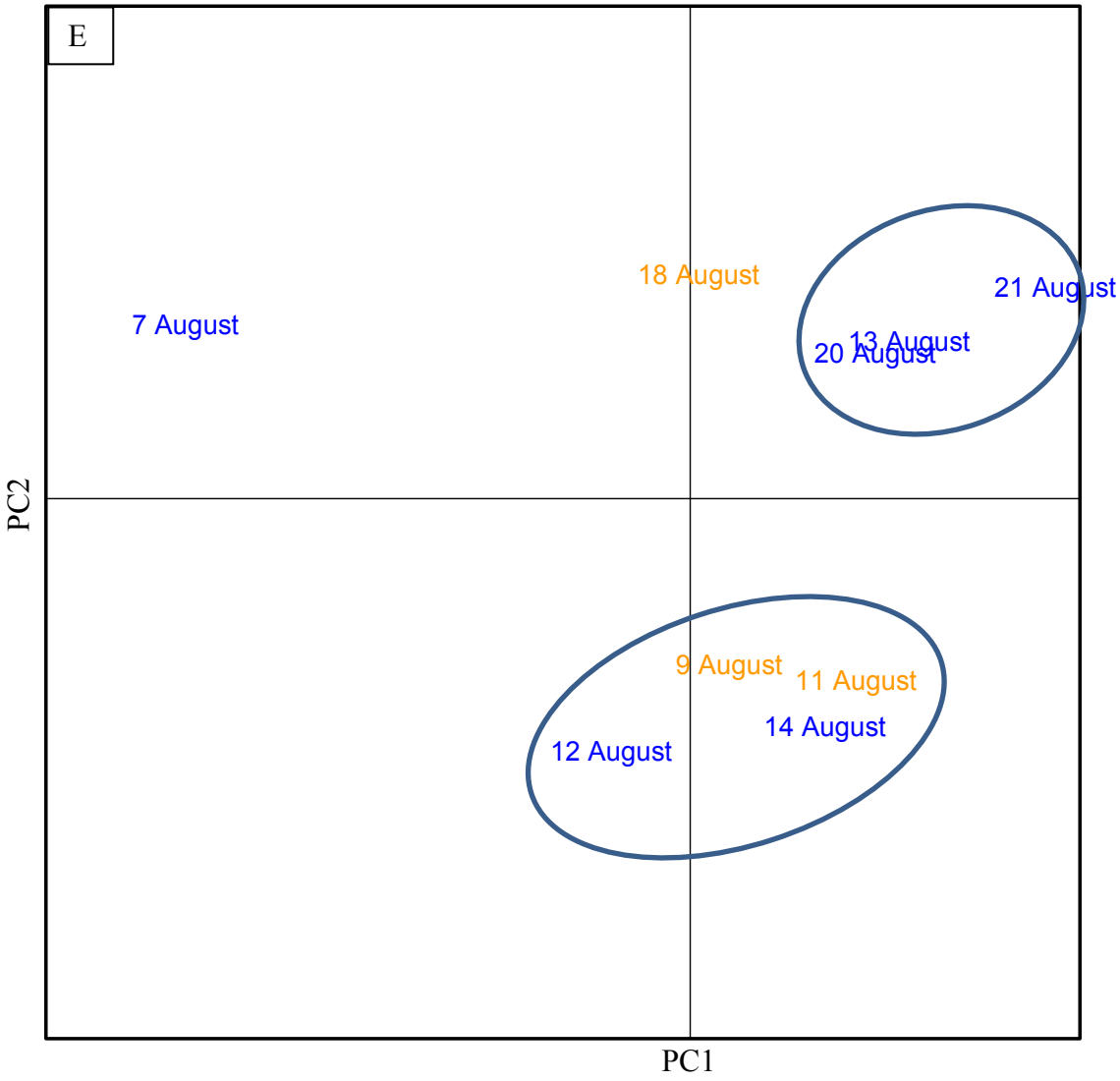
Legend
Dead
0% of frames full of pollen
Less than 1% of frames full of pollen
1-5% of frames full of pollen
5.01-10% of frames full of pollen
10.01-15% of frames full of pollen
15.01-20% of frames full of pollen
20.01-25% of frames full of pollen
25.01-30% of frames full of pollen



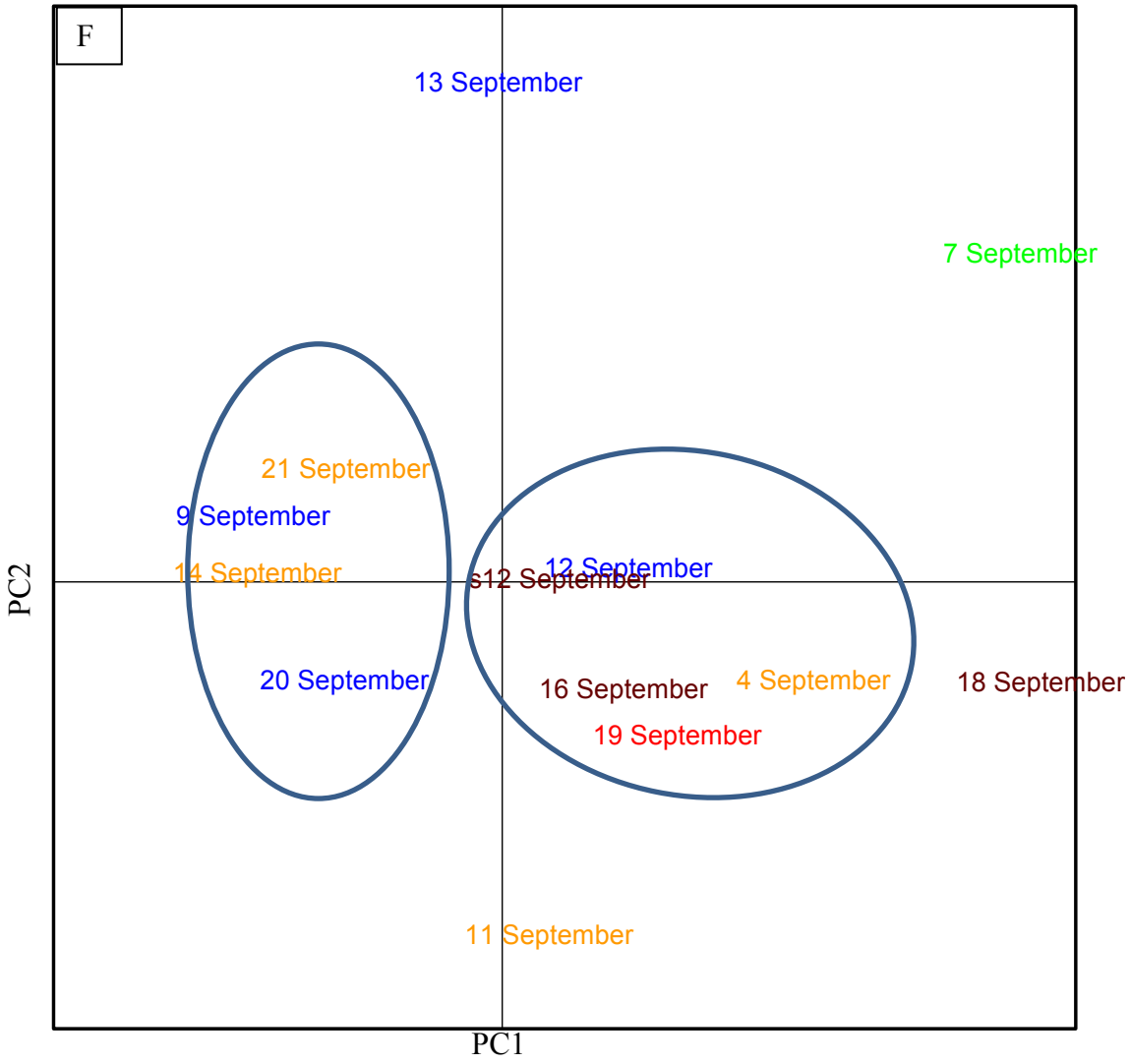
Legend
Dead
0% of frames full of pollen
Less than 1% of frames full of pollen
1-5% of frames full of pollen
5.01-10% of frames full of pollen
10.01-15% of frames full of pollen
15.01-20% of frames full of pollen
20.01-25% of frames full of pollen
25.01-30% of frames full of pollen



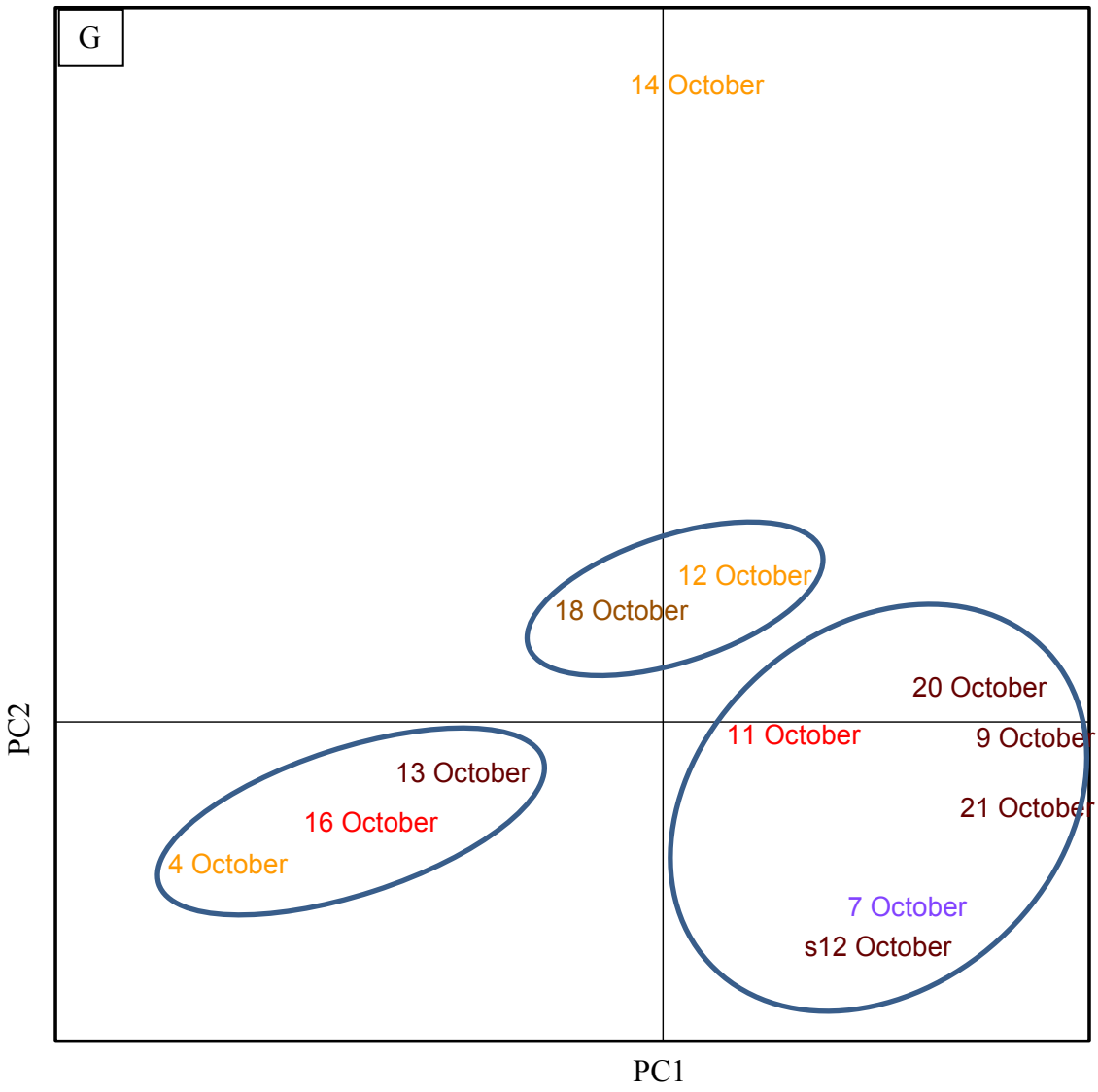
Legend
Dead
0% of frames full of pollen
Less than 1% of frames full of pollen
1-5% of frames full of pollen
5.01-10% of frames full of pollen
10.01-15% of frames full of pollen
15.01-20% of frames full of pollen
20.01-25% of frames full of pollen
25.01-30% of frames full of pollen



Legend
Dead
0% of frames full of pollen
Less than 1% of frames full of pollen
1-5% of frames full of pollen
5.01-10% of frames full of pollen
10.01-15% of frames full of pollen
15.01-20% of frames full of pollen
20.01-25% of frames full of pollen
25.01-30% of frames full of pollen



Legend
Dead
0% of frames full of pollen
Less than 1% of frames full of pollen
1-5% of frames full of pollen
5.01-10% of frames full of pollen
10.01-15% of frames full of pollen
15.01-20% of frames full of pollen
20.01-25% of frames full of pollen
25.01-30% of frames full of pollen

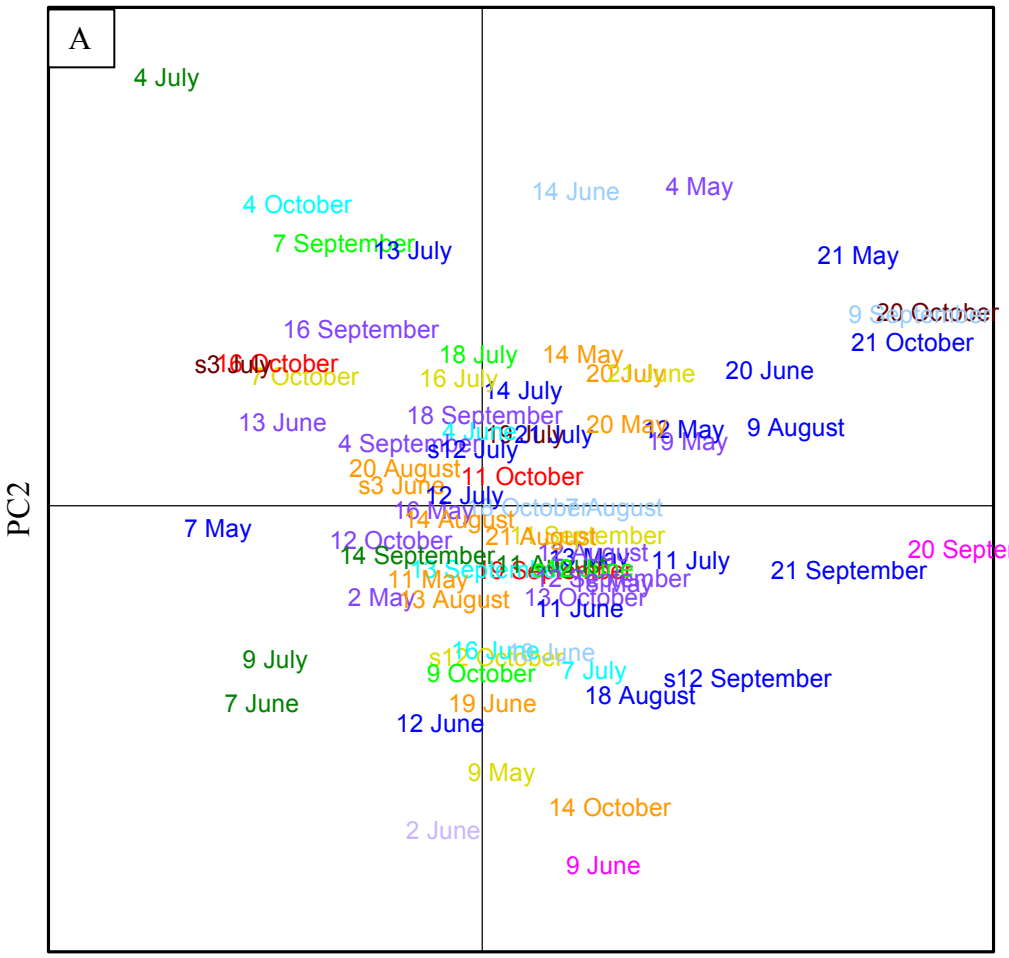


Legend
Dead
0% of frames full of pollen
Less than 1% of frames full of pollen
1-5% of frames full of pollen
5.01-10% of frames full of pollen
10.01-15% of frames full of pollen
15.01-20% of frames full of pollen
20.01-25% of frames full of pollen
25.01-30% of frames full of pollen

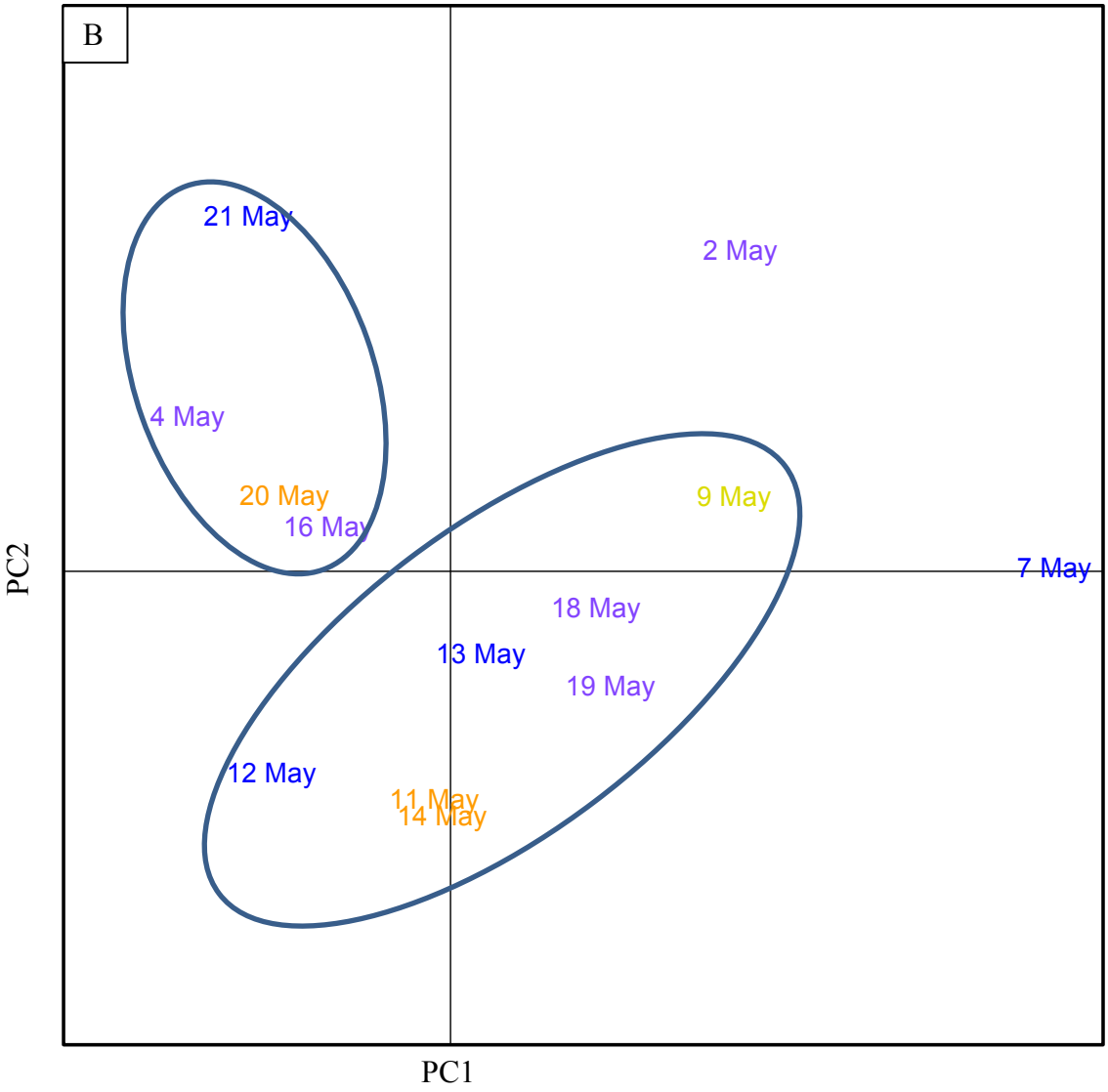
Figure E1 A-G: PCA of all DGGE bacterial profiles of bee bread sampled monthly from the South Campus Research Apiary by the amount of pollen. Preceding numbers indicate which colony the bee bread was sampled from and the following month indicates the month the bee bread was sampled. A.) 2D PCA of all bee bread samples. The first and second components represent 5.4% and 5.0% of the variation respectively. B.) 2D PCA of May bee bread samples. The first and second components represent 16.8% and 12.8% of the variation respectively. C.) 2D PCA of June bee bread samples. The first and second components represent 15.2% and 11.3% of the variation respectively. D.) 2D PCA of July bee bread samples. The first and second components represent 14.9% and 12.8% of the variation respectively. E.) 2D PCA of August bee bread samples. The first and second components represent 19.4% and 16.5% of the variation respectively. F.) 2D PCA of September bee bread samples. The first and and second components represents 14.6% and 12.2% of the variation respectively. G.) 2D PCA of October bee bread samples. The first and second components represent 17.3% and 14.9% of the variation respectively.

APPENDIX F

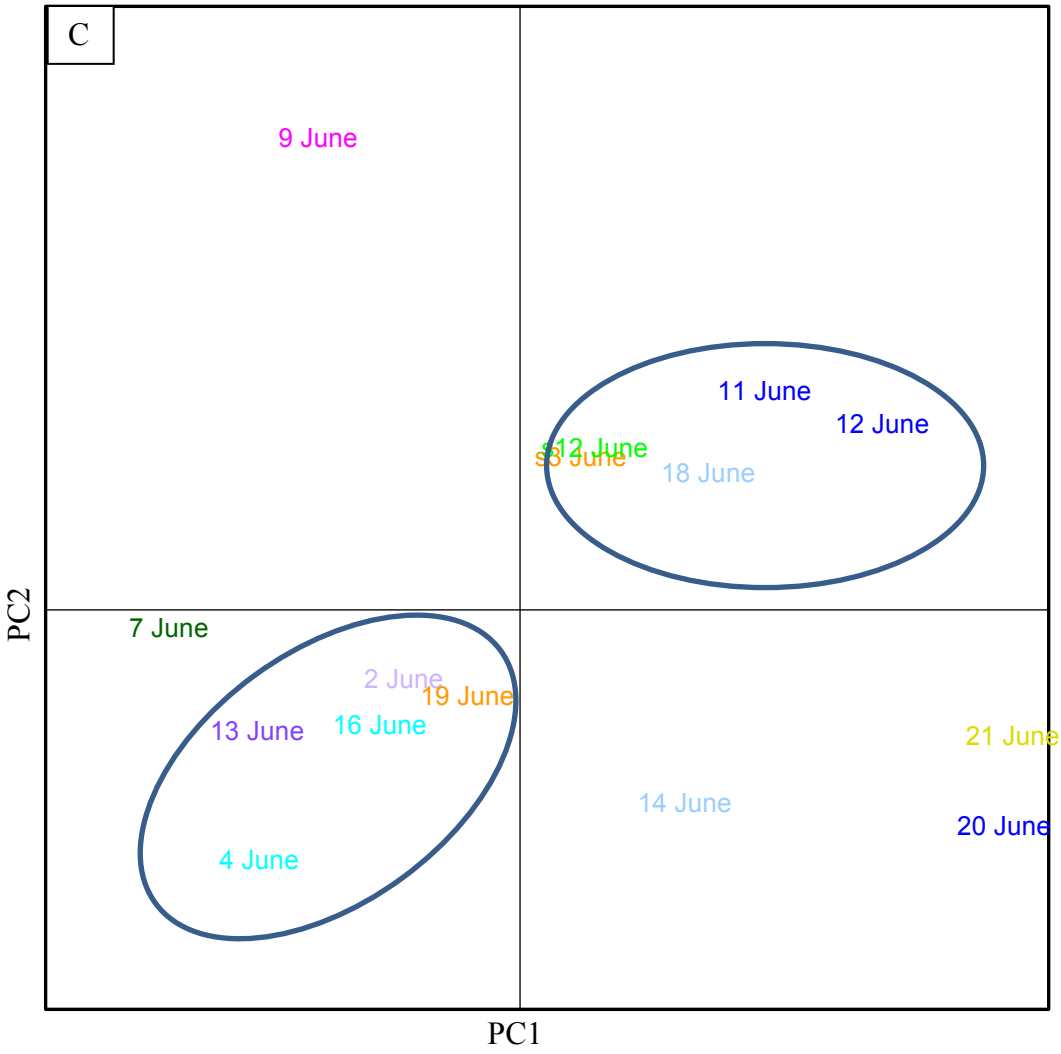
PCA OF ALL BEE BREAD BACTERIAL PROFILES BY THE AMOUNT OF NECTAR



Legend
Dead
0% of frames full of nectar
Less than 1% of frames full of nectar
1-5% of frames full of nectar
5.01-10% of frames full of nectar
10.01-15% of frames full of nectar
15.01-20% of frames full of nectar
20.01-25% of frames full of nectar
25.01-30% of frames full of nectar
30.01-35% of frames full of nectar
35.01-40% of frames full of nectar
40.01-45% of frames full of nectar
45.01-50% of frames full of nectar
50.01-55% of frames full of nectar



Legend
Dead
0% of frames full of nectar
Less than 1% of frames full of nectar
1-5% of frames full of nectar
5.01-10% of frames full of nectar
10.01-15% of frames full of nectar
15.01-20% of frames full of nectar
20.01-25% of frames full of nectar
25.01-30% of frames full of nectar
30.01-35% of frames full of nectar
35.01-40% of frames full of nectar
40.01-45% of frames full of nectar
45.01-50% of frames full of nectar
50.01-55% of frames full of nectar



Legend
Dead
0% of frames full of nectar
Less than 1% of frames full of nectar
1-5% of frames full of nectar
5.01-10% of frames full of nectar
10.01-15% of frames full of nectar
15.01-20% of frames full of nectar
20.01-25% of frames full of nectar
25.01-30% of frames full of nectar
30.01-35% of frames full of nectar
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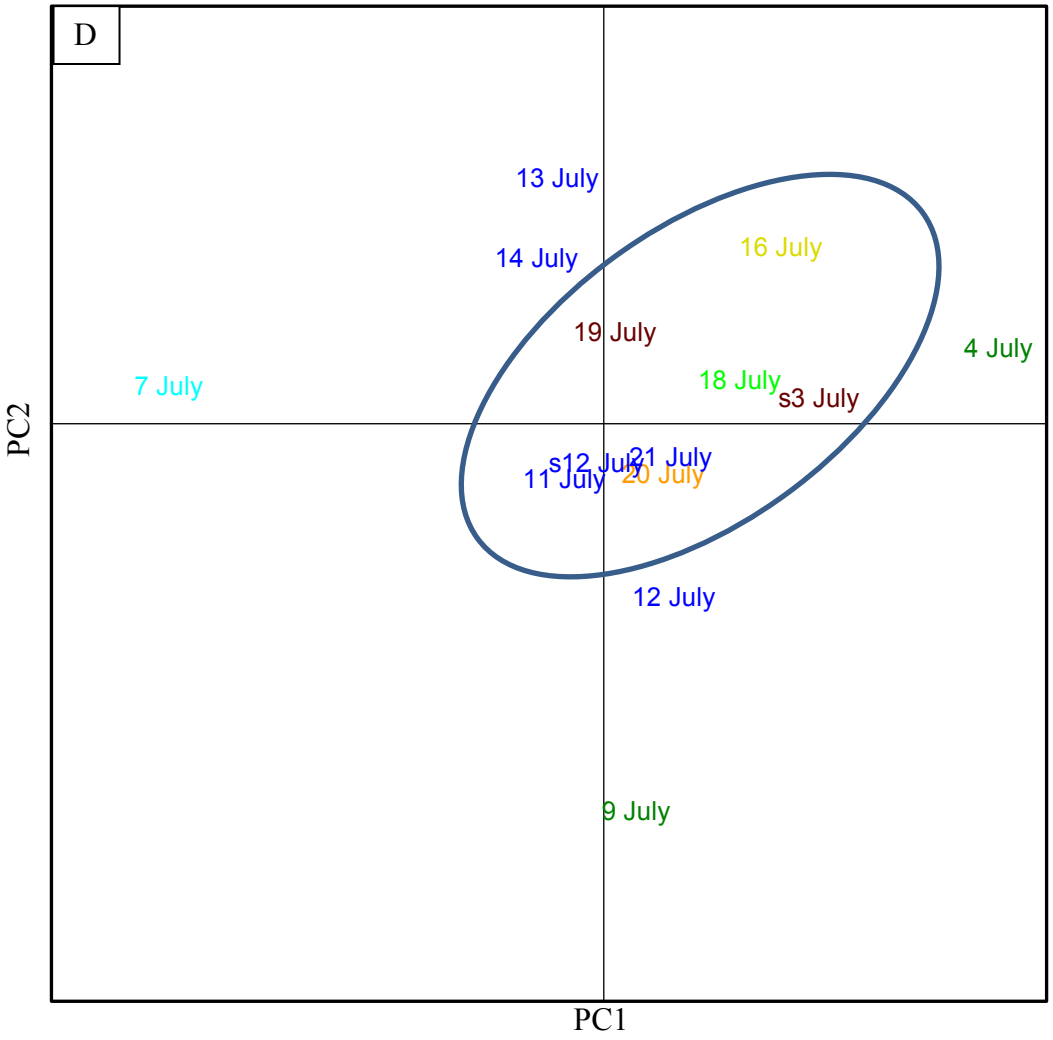
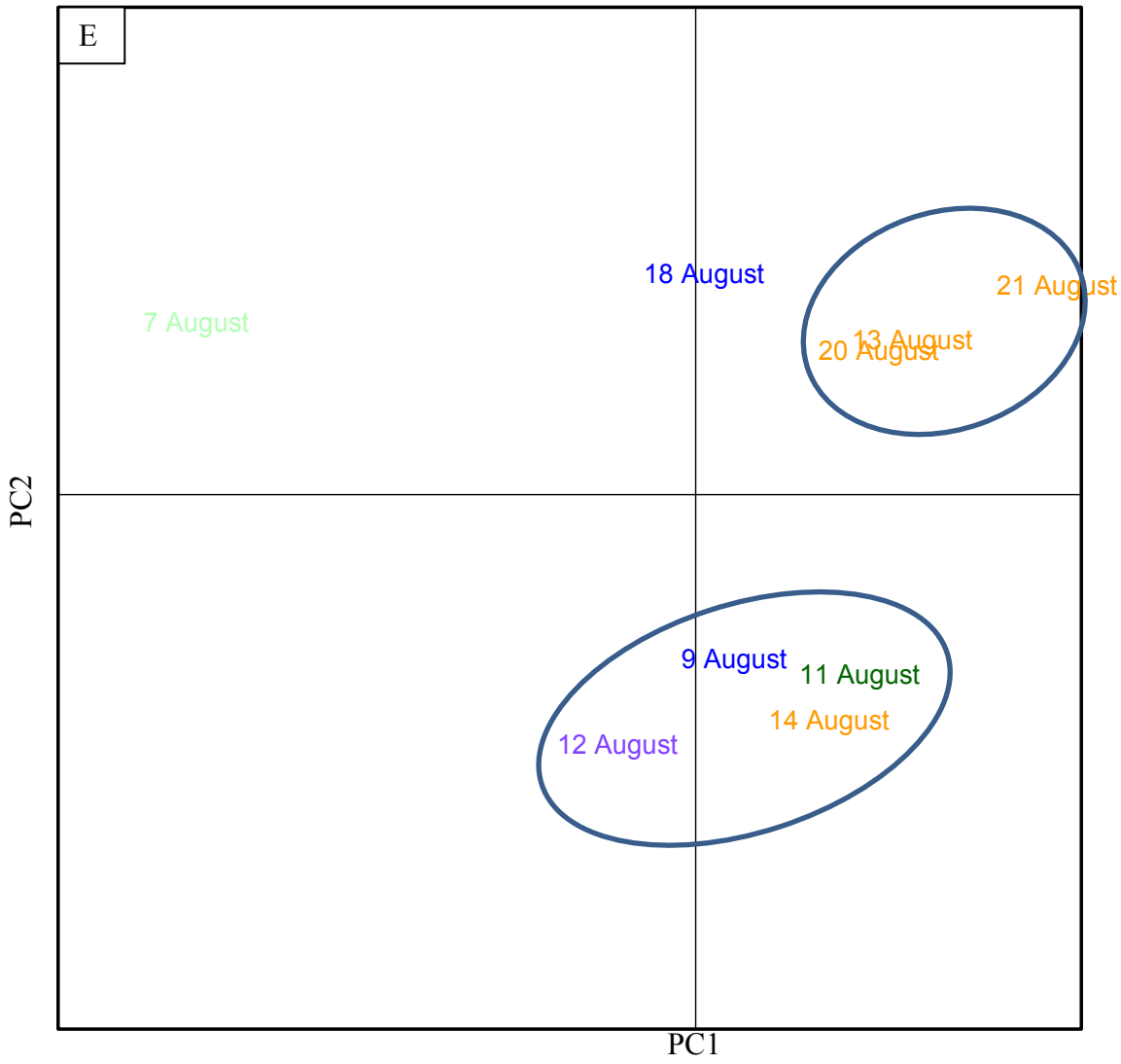
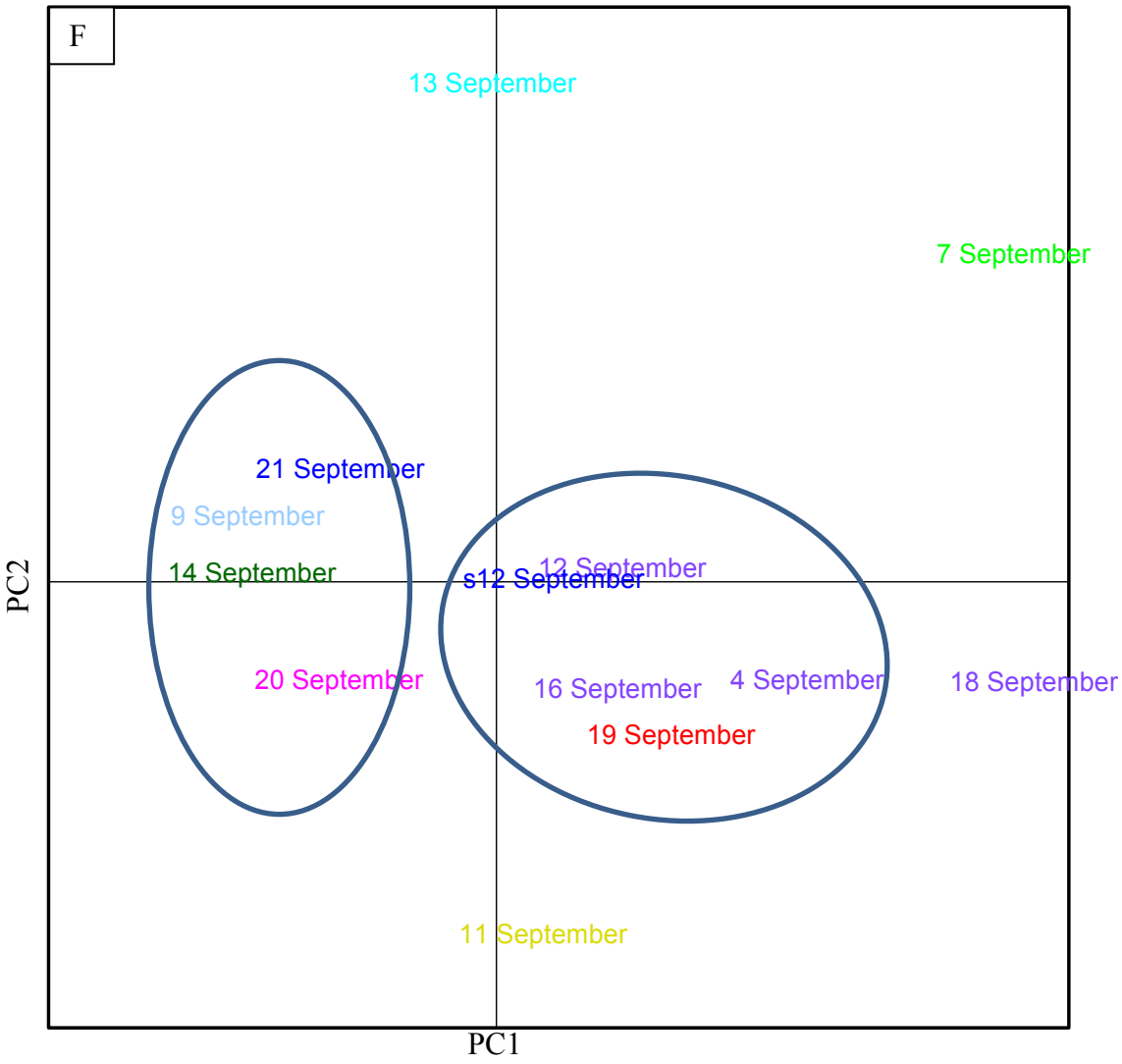
45.01-50% of frames full of nectar
50.01-55% of frames full of nectar



Legend
Dead
0% of frames full of nectar
Less than 1% of frames full of nectar
1-5% of frames full of nectar
5.01-10% of frames full of nectar
10.01-15% of frames full of nectar
15.01-20% of frames full of nectar
20.01-25% of frames full of nectar
25.01-30% of frames full of nectar
30.01-35% of frames full of nectar
35.01-40% of frames full of nectar
40.01-45% of frames full of nectar
45.01-50% of frames full of nectar
50.01-55% of frames full of nectar



Legend
Dead
0% of frames full of nectar
Less than 1% of frames full of nectar
1-5% of frames full of nectar
5.01-10% of frames full of nectar
10.01-15% of frames full of nectar
15.01-20% of frames full of nectar
20.01-25% of frames full of nectar
25.01-30% of frames full of nectar
30.01-35% of frames full of nectar
35.01-40% of frames full of nectar
40.01-45% of frames full of nectar
45.01-50% of frames full of nectar
50.01-55% of frames full of nectar



Legend
Dead
0% of frames full of nectar
Less than 1% of frames full of nectar
1-5% of frames full of nectar
5.01-10% of frames full of nectar
10.01-15% of frames full of nectar
15.01-20% of frames full of nectar
20.01-25% of frames full of nectar
25.01-30% of frames full of nectar
30.01-35% of frames full of nectar
35.01-40% of frames full of nectar
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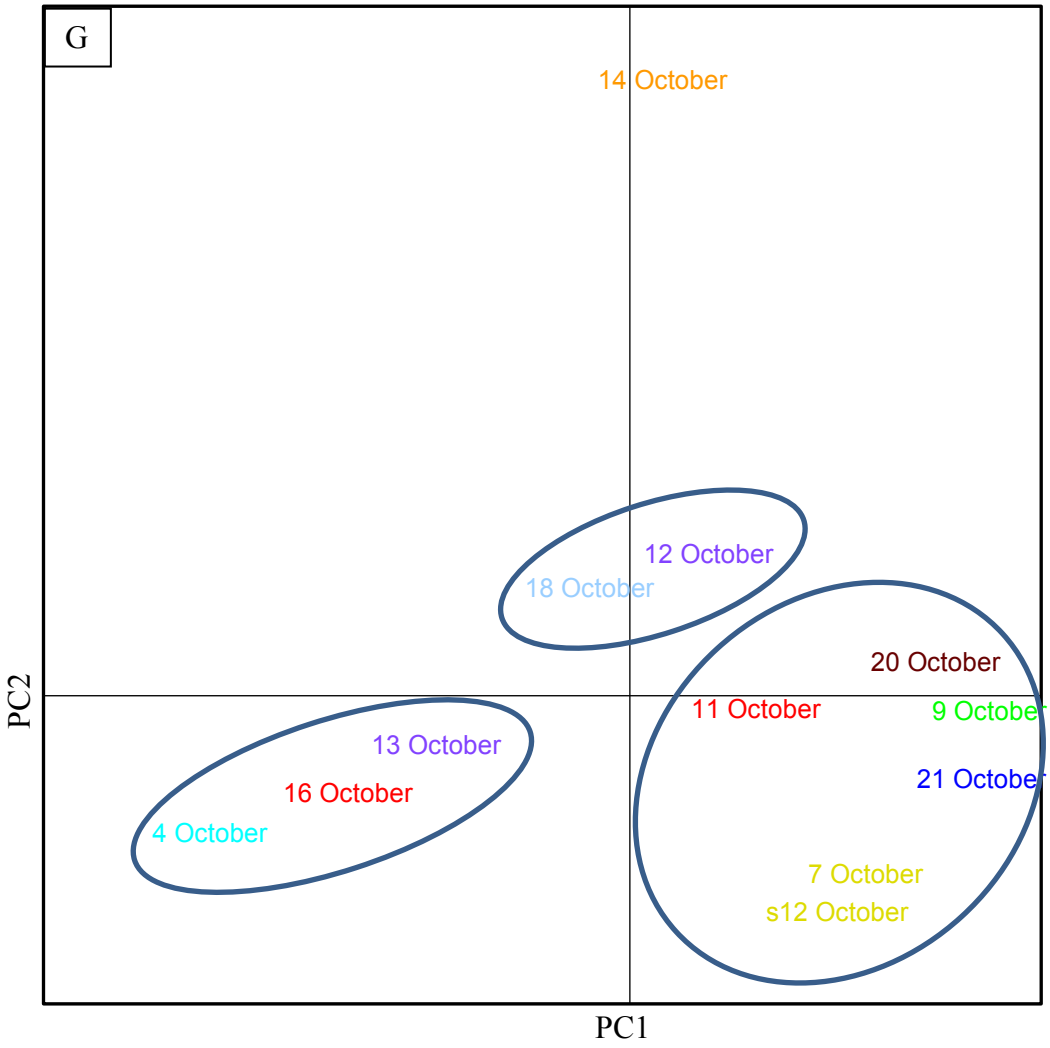
50.01-55% of frames full of nectar

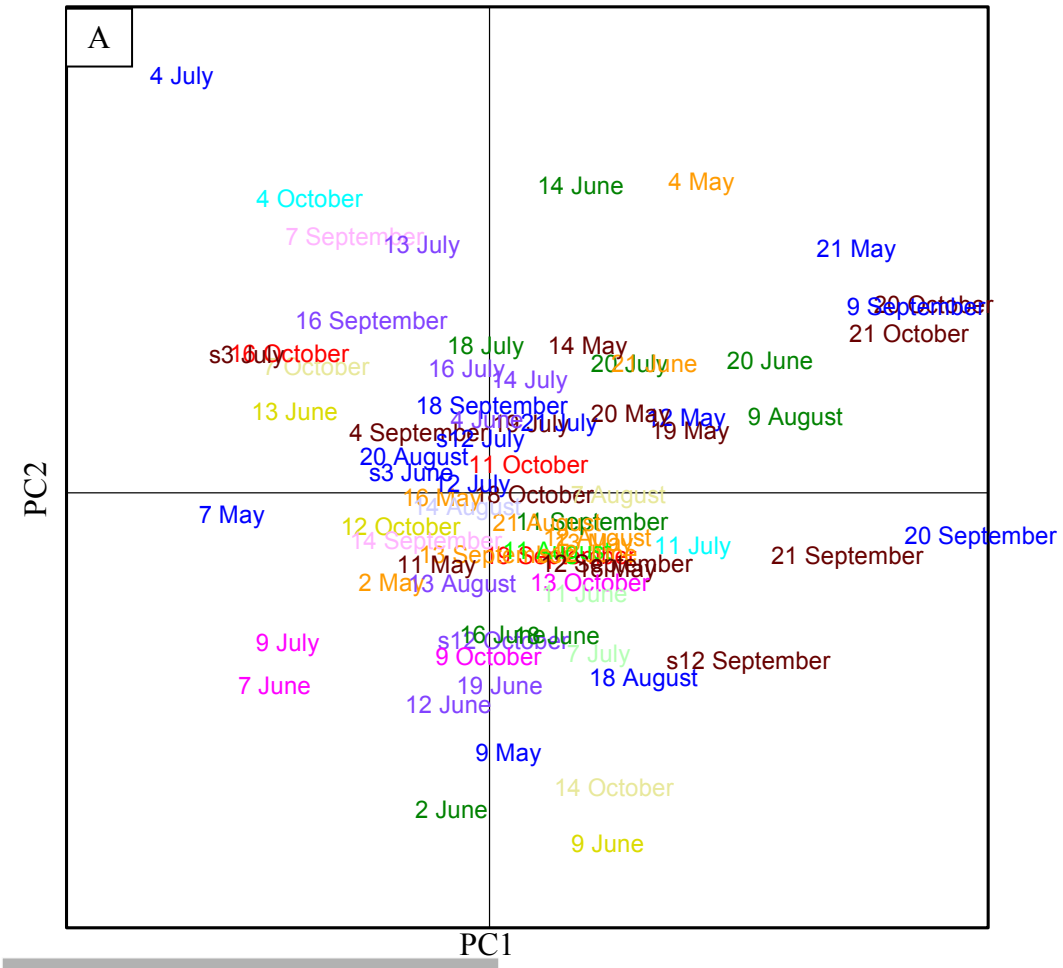


Legend
Dead
0% of frames full of nectar
Less than 1% of frames full of nectar
1-5% of frames full of nectar
5.01-10% of frames full of nectar
10.01-15% of frames full of nectar
15.01-20% of frames full of nectar
20.01-25% of frames full of nectar
25.01-30% of frames full of nectar
30.01-35% of frames full of nectar
35.01-40% of frames full of nectar
40.01-45% of frames full of nectar
45.01-50% of frames full of nectar
50.01-55% of frames full of nectar

Figure F1 A-G: PCA of all DGGE bacterial profiles of bee bread sampled monthly from the South Campus Research Apiary by the amount of nectar. Preceding numbers indicate which colony the bee bread was sampled from and the following month indicates the month the bee bread was sampled. A.) 2D PCA of all bee bread samples. The first and second components represent 5.4% and 5.0% of the variation respectively. B.) 2D PCA of May bee bread samples. The first and second components represent 16.8% and 12.8% of the variation respectively. C.) 2D PCA of June bee bread samples. The first and second components represent 15.2% and 11.3% of the variation respectively. D.) 2D PCA of July bee bread samples. The first and second components represent 14.9% and 12.8% of the variation respectively. E.) 2D PCA of August bee bread samples. The first and second components represent 19.4% and 16.5% of the variation respectively. F.) 2D PCA of September bee bread samples. The first and and second components represents 14.6% and 12.2% of the variation respectively. G.) 2D PCA of October bee bread samples. The first and second components represent 17.3% and 14.9% of the variation respectively.

APPENDIX G

PCA OF ALL BEE BREAD BACTERIAL PROFILES BY THE AMOUNT OF HONEY



Legend
Dead
0% of frames full of honey
Less than 1% of frames full of honey
1-5% of frames full of honey
5.01-10% of frames full of honey
10.01-15% of frames full of honey
15.01-20% of frames full of honey
20.01-25% of frames full of honey
25.01-30% of frames full of honey
30.01-35% of frames full of honey
35.01-40% of frames full of honey
40.01-45% of frames full of honey
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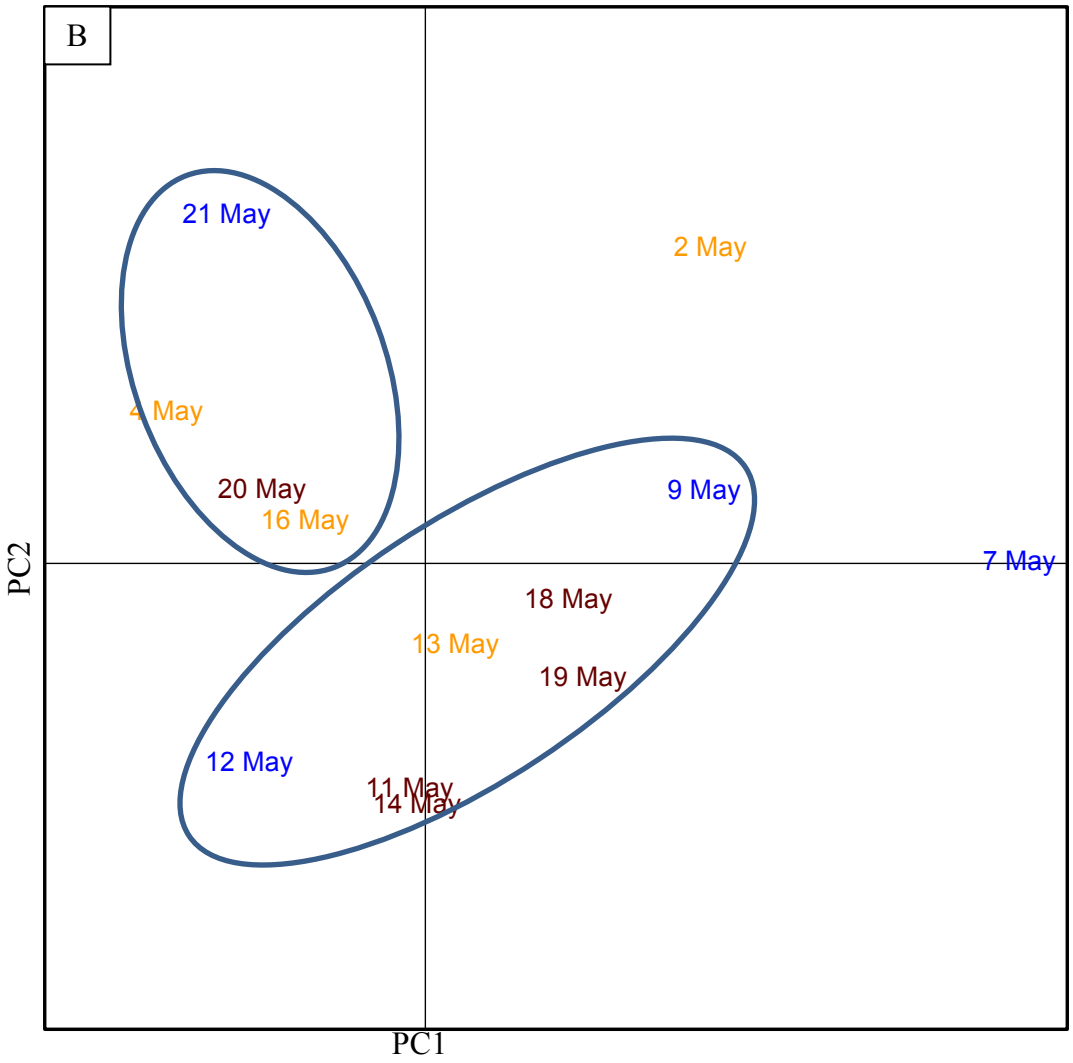
55.01-60% of frames full of honey
60.01-65% of frames full of honey



Legend
Dead
0% of frames full of honey
Less than 1% of frames full of honey
1-5% of frames full of honey
5.01-10% of frames full of honey
10.01-15% of frames full of honey
15.01-20% of frames full of honey
20.01-25% of frames full of honey
25.01-30% of frames full of honey
30.01-35% of frames full of honey
35.01-40% of frames full of honey
40.01-45% of frames full of honey
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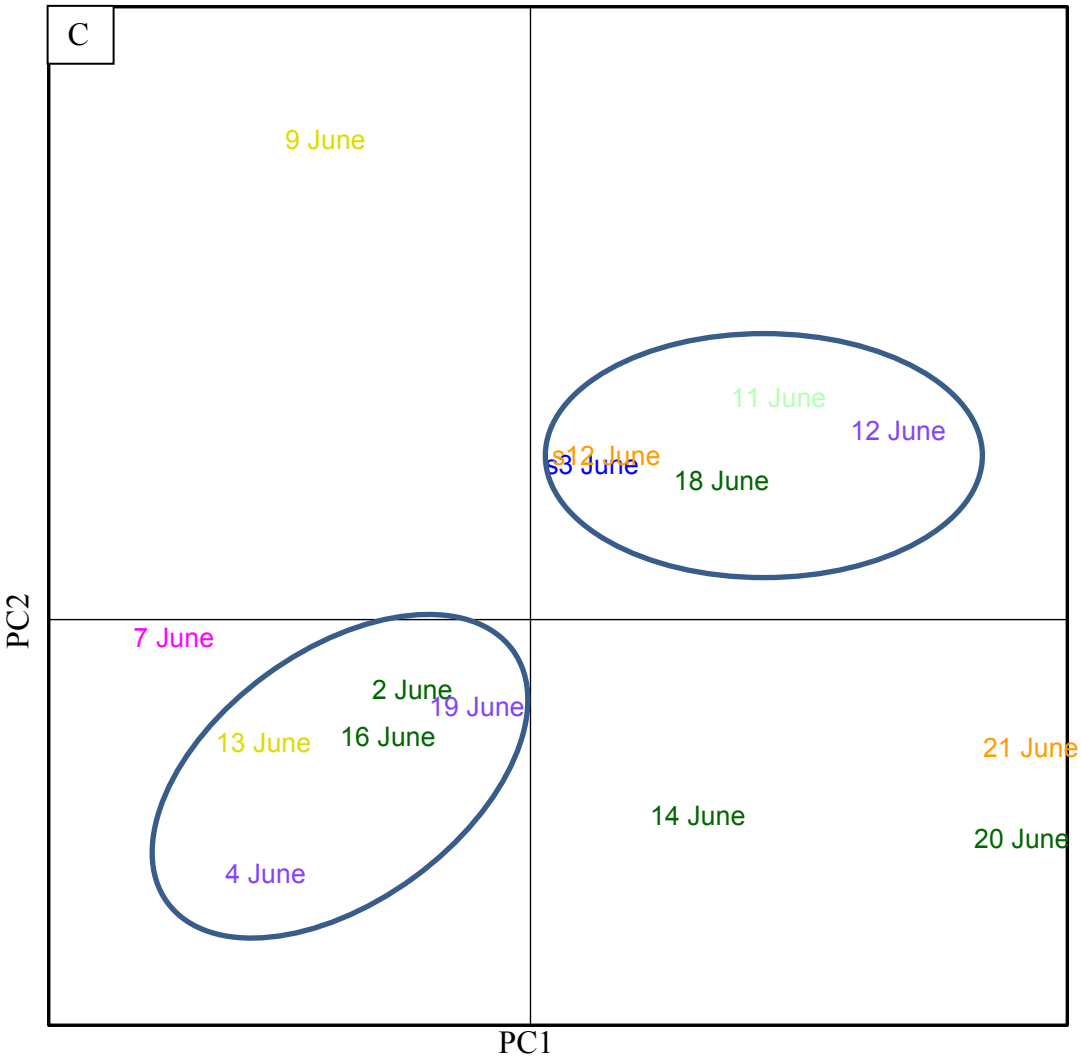
55.01-60% of frames full of honey
60.01-65% of frames full of honey



Legend
Dead
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Less than 1% of frames full of honey
1-5% of frames full of honey
5.01-10% of frames full of honey
10.01-15% of frames full of honey
15.01-20% of frames full of honey
20.01-25% of frames full of honey
25.01-30% of frames full of honey
30.01-35% of frames full of honey
35.01-40% of frames full of honey
40.01-45% of frames full of honey
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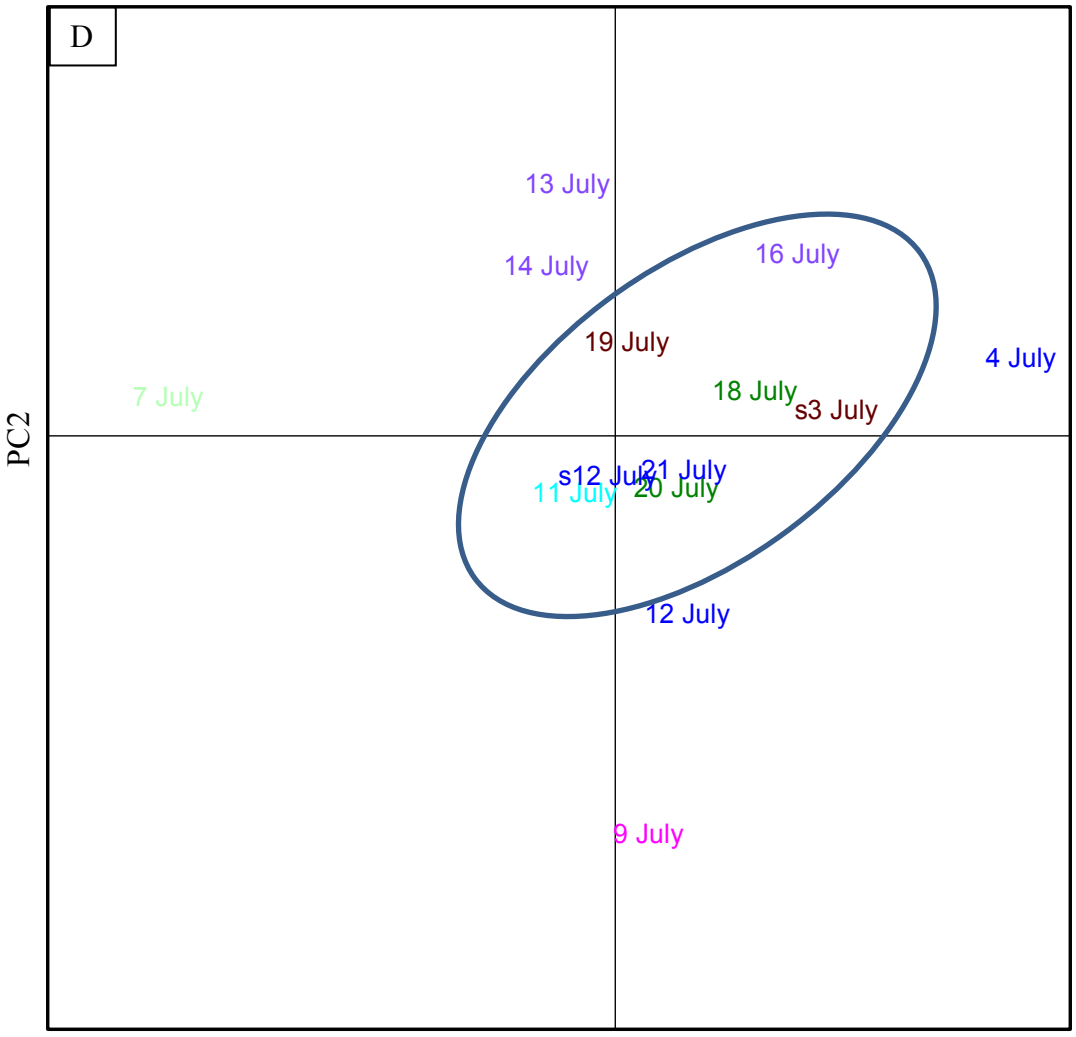
60.01-65% of frames full of honey



Legend
Dead
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Less than 1% of frames full of honey
1-5% of frames full of honey
5.01-10% of frames full of honey
10.01-15% of frames full of honey
15.01-20% of frames full of honey
20.01-25% of frames full of honey
25.01-30% of frames full of honey
30.01-35% of frames full of honey
35.01-40% of frames full of honey
40.01-45% of frames full of honey
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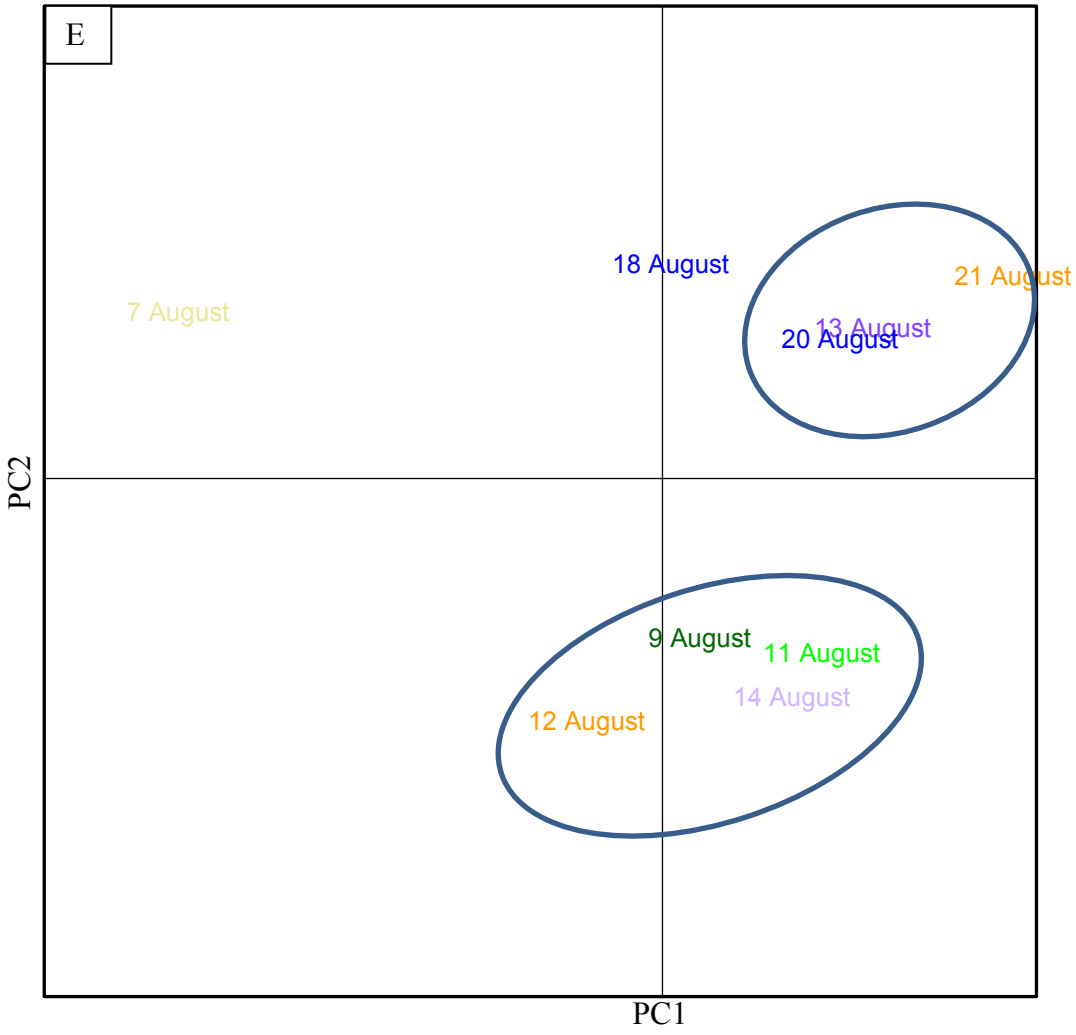
55.01-60% of frames full of honey
60.01-65% of frames full of honey



Legend
Dead
0% of frames full of honey
Less than 1% of frames full of honey
1-5% of frames full of honey
5.01-10% of frames full of honey
10.01-15% of frames full of honey
15.01-20% of frames full of honey
20.01-25% of frames full of honey
25.01-30% of frames full of honey
30.01-35% of frames full of honey
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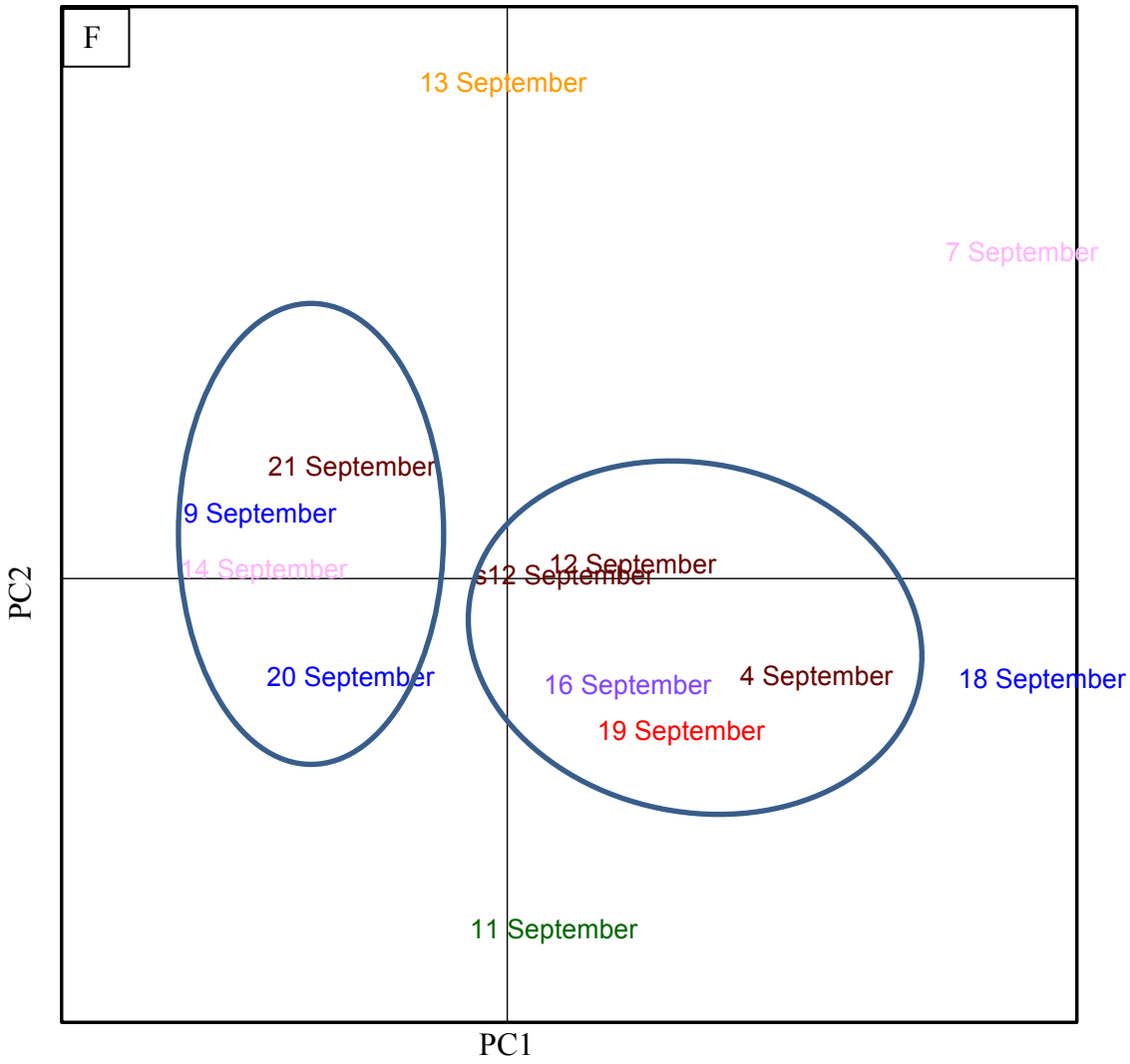
60.01-65% of frames full of honey



Legend
Dead
0% of frames full of honey
Less than 1% of frames full of honey
1-5% of frames full of honey
5.01-10% of frames full of honey
10.01-15% of frames full of honey
15.01-20% of frames full of honey
20.01-25% of frames full of honey
25.01-30% of frames full of honey
30.01-35% of frames full of honey
35.01-40% of frames full of honey
40.01-45% of frames full of honey
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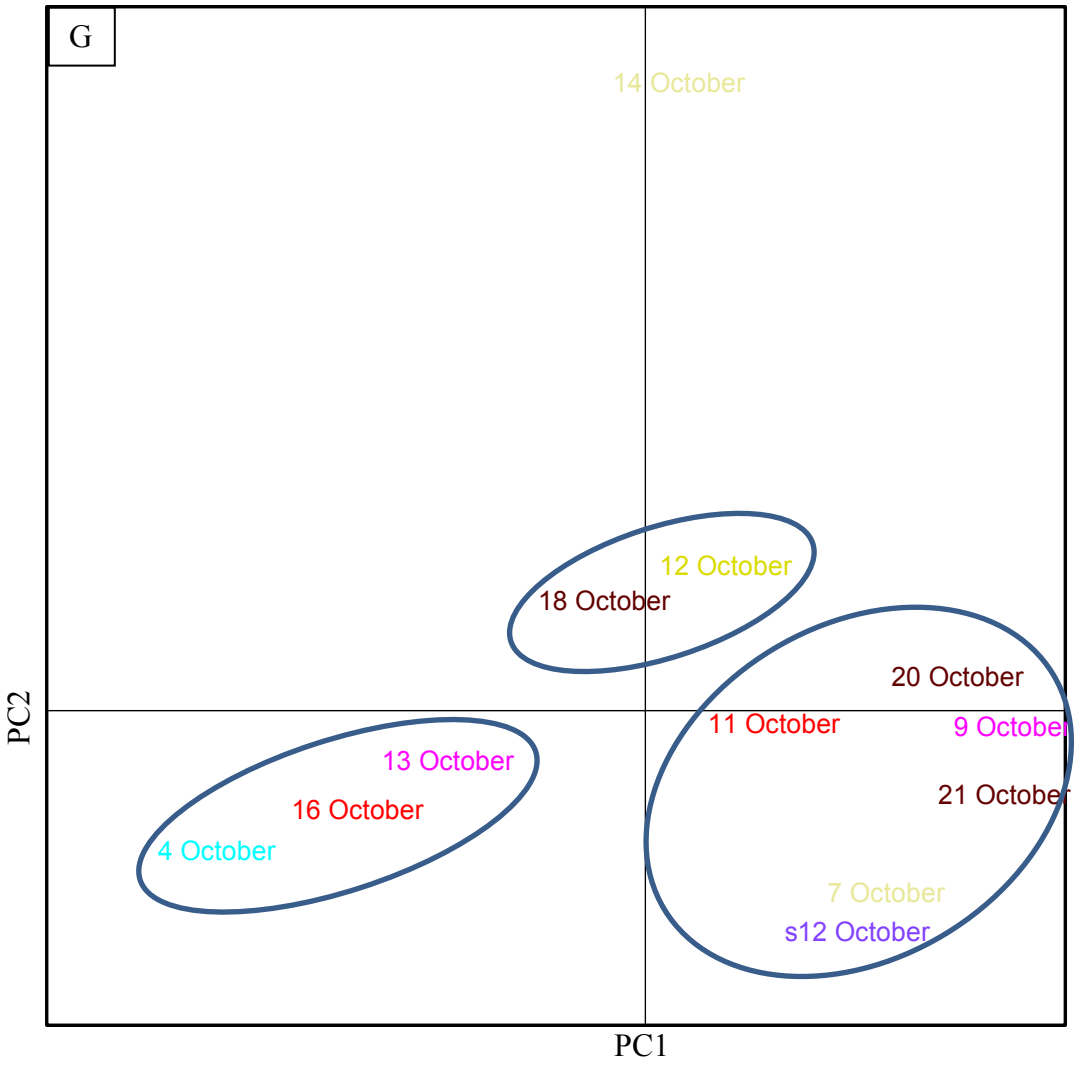
60.01-65% of frames full of honey

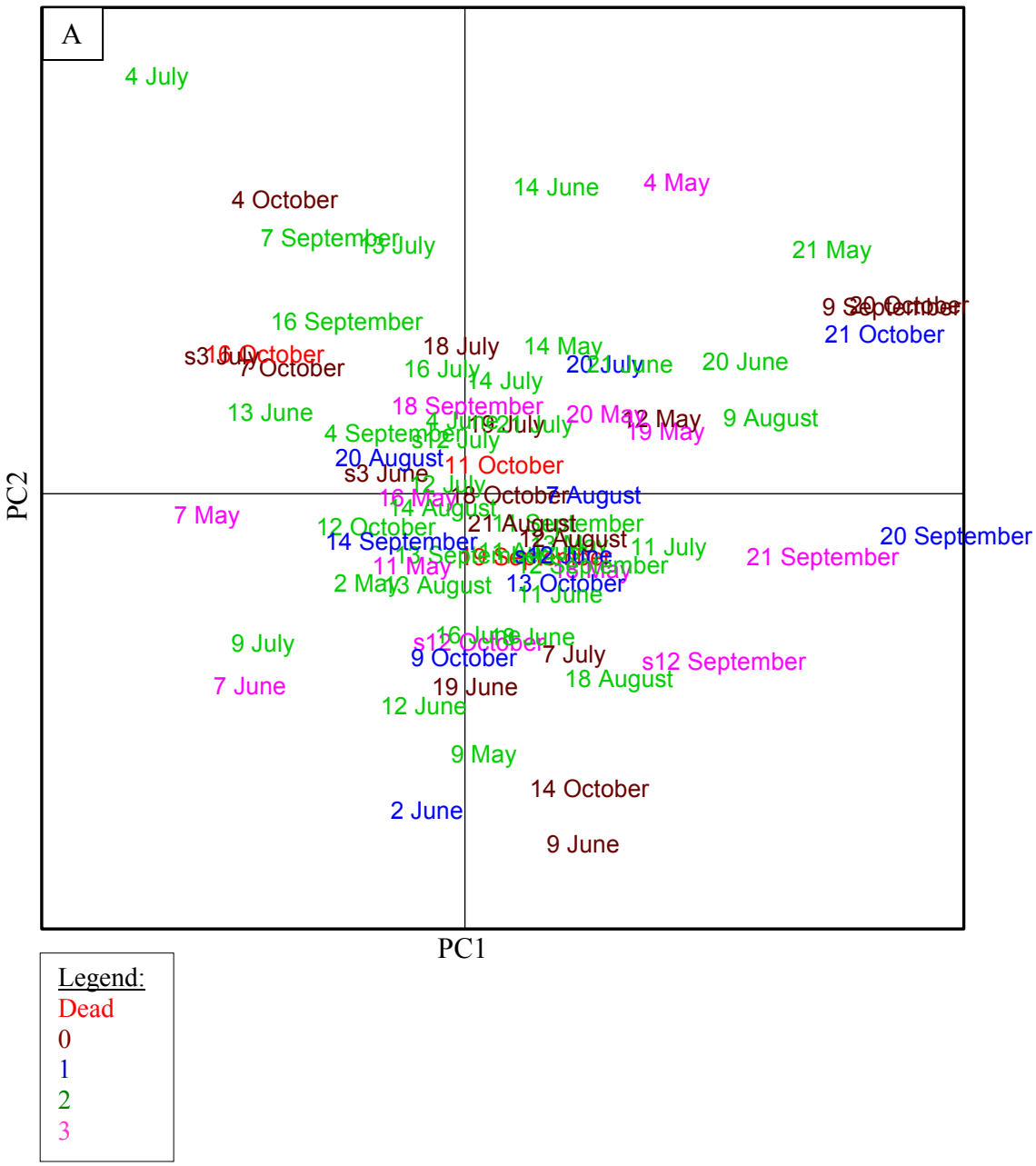


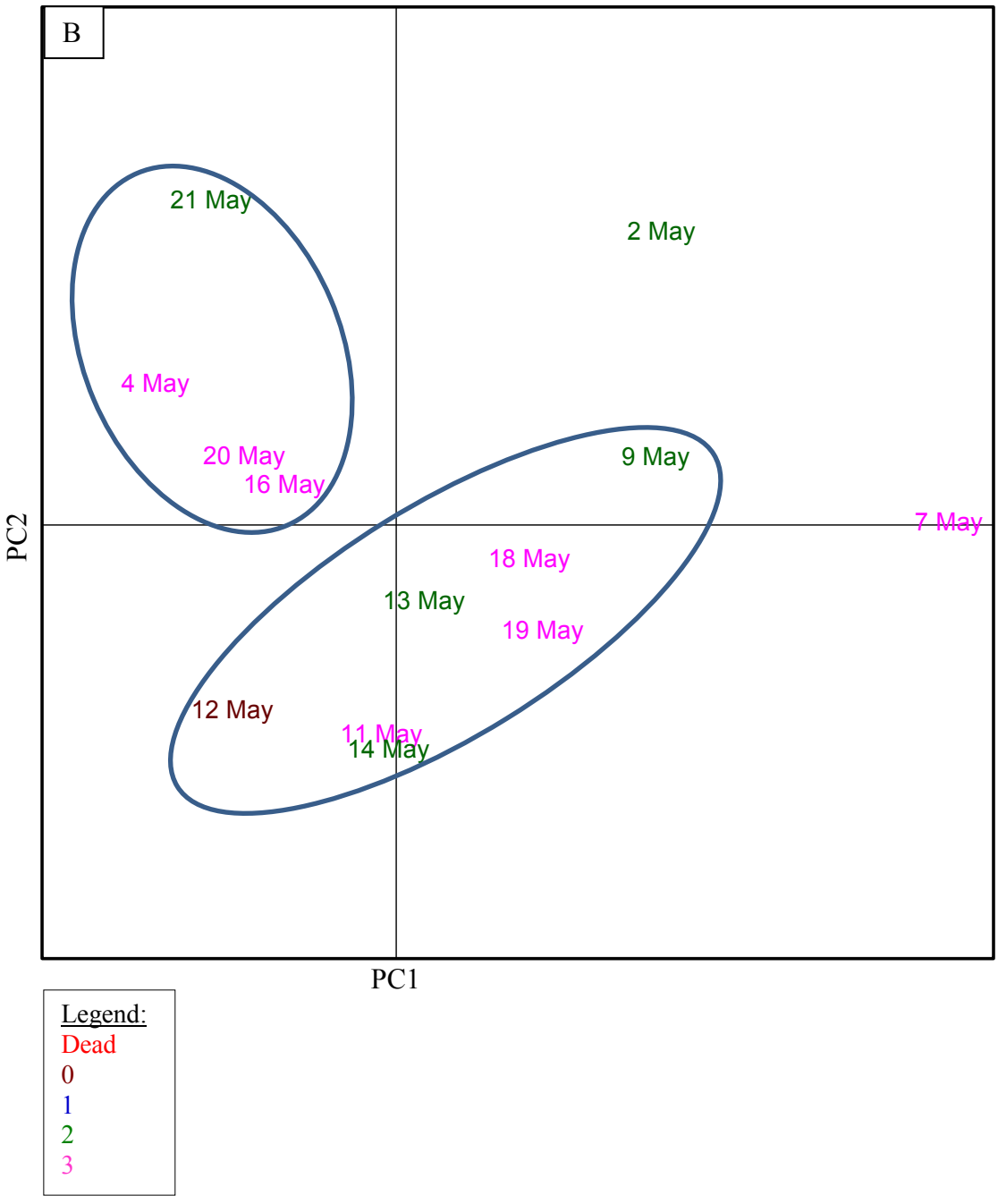
Legend
Dead
0% of frames full of honey
Less than 1% of frames full of honey
1-5% of frames full of honey
5.01-10% of frames full of honey
10.01-15% of frames full of honey
15.01-20% of frames full of honey
20.01-25% of frames full of honey
25.01-30% of frames full of honey
30.01-35% of frames full of honey
35.01-40% of frames full of honey
40.01-45% of frames full of honey
45.01-50% of frames full of honey
50.01-55% of frames full of honey
55.01-60% of frames full of honey
60.01-65% of frames full of honey

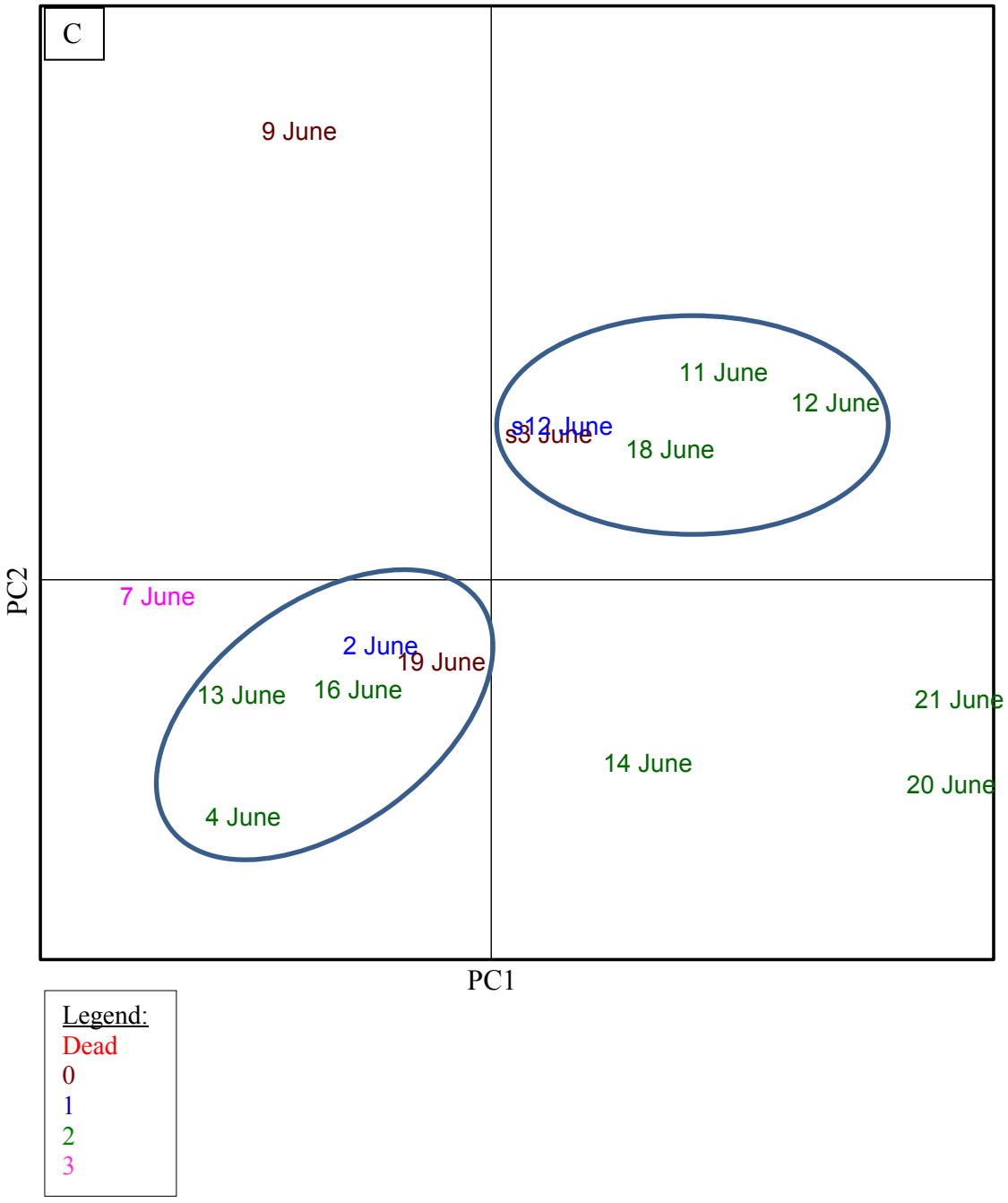
Figure G1 A-G: PCA of all DGGE bacterial profiles of bee bread sampled monthly from the South Campus Research Apiary by the amount of honey. Preceding numbers indicate which colony the bee bread was sampled from and the following month indicates the month the bee bread was sampled A.) 2D PCA of all bee bread samples. The first and second components represent 5.4% and 5.0% of the variation respectively. B.) 2D PCA of May bee bread samples. The first and second components represent 16.8% and 12.8% of the variation respectively. C.) 2D PCA of June bee bread samples. The first and second components represent 15.2% and 11.3% of the variation respectively. D.) 2D PCA of July bee bread samples. The first and second components represent 14.9% and 12.8% of the variation respectively. E.) 2D PCA of August bee bread samples. The first and second components represent 19.4% and 16.5% of the variation respectively. F.) 2D PCA of September bee bread samples. The first and and second components represents 14.6% and 12.2% of the variation respectively. G.) 2D PCA of October bee bread samples. The first and second components represent 17.3% and 14.9% of the variation respectively.

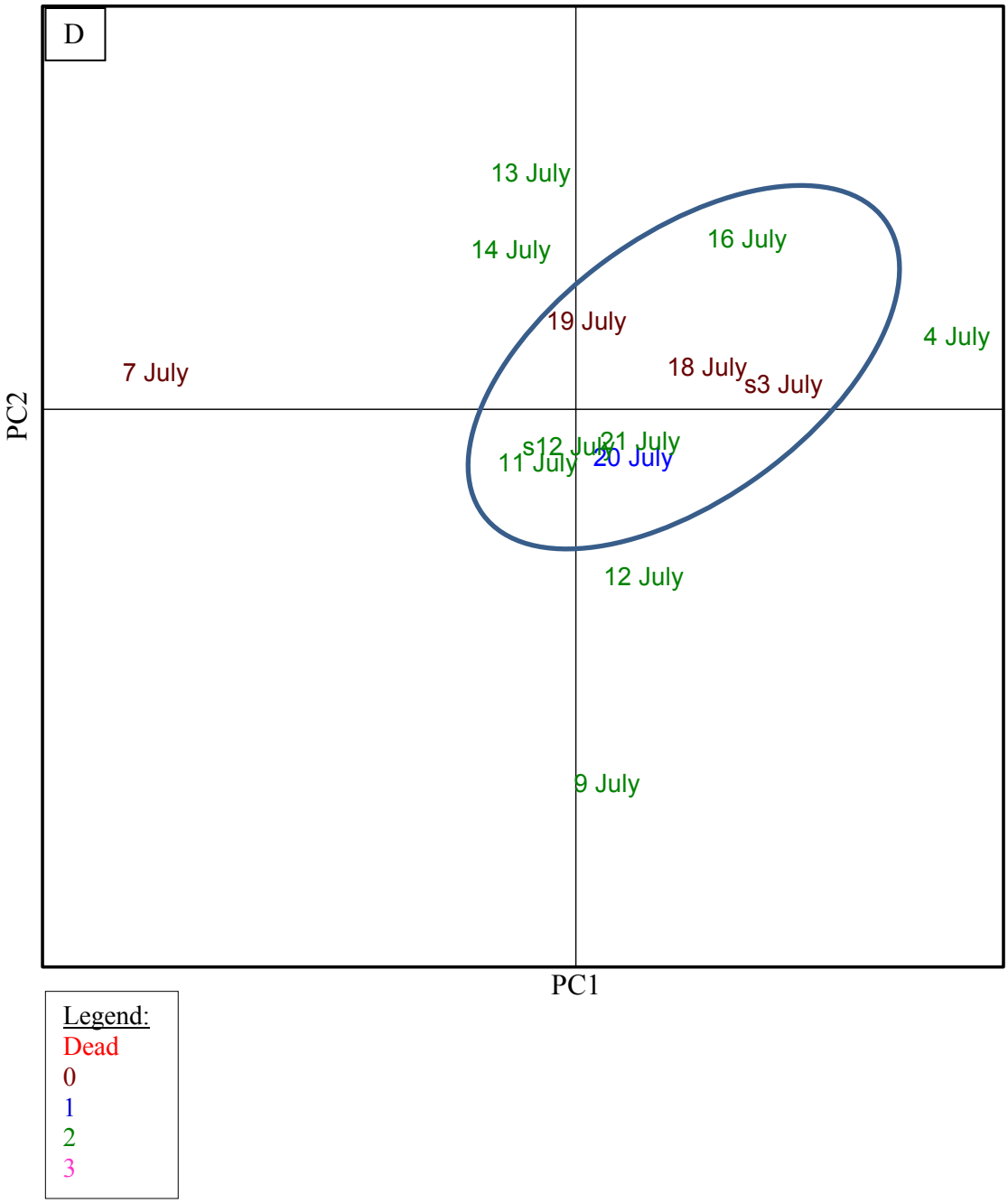
APPENDIX H

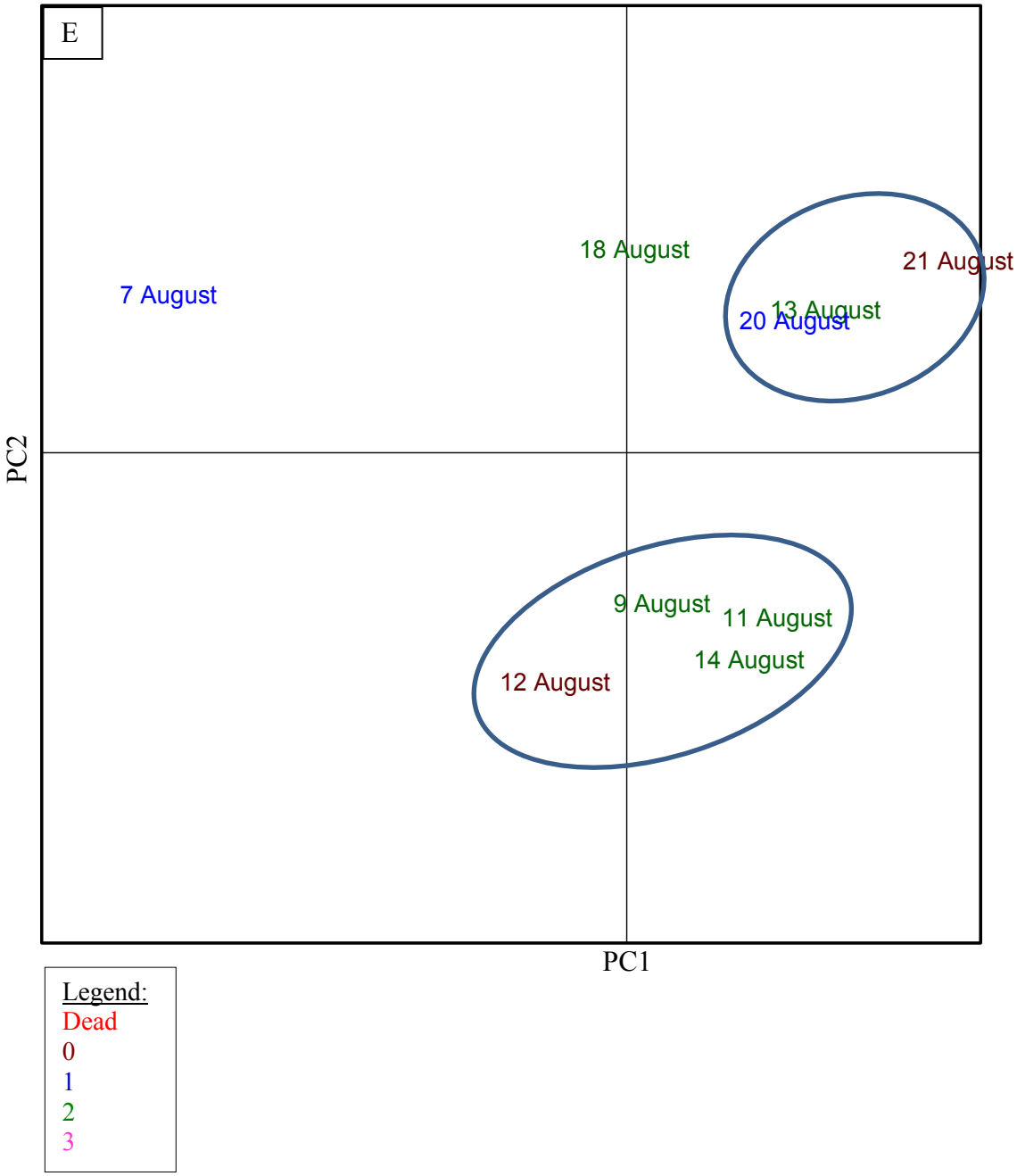
PCA OF ALL BEE BREAD BACTERIAL PROFILES BY BROOD PATTERN

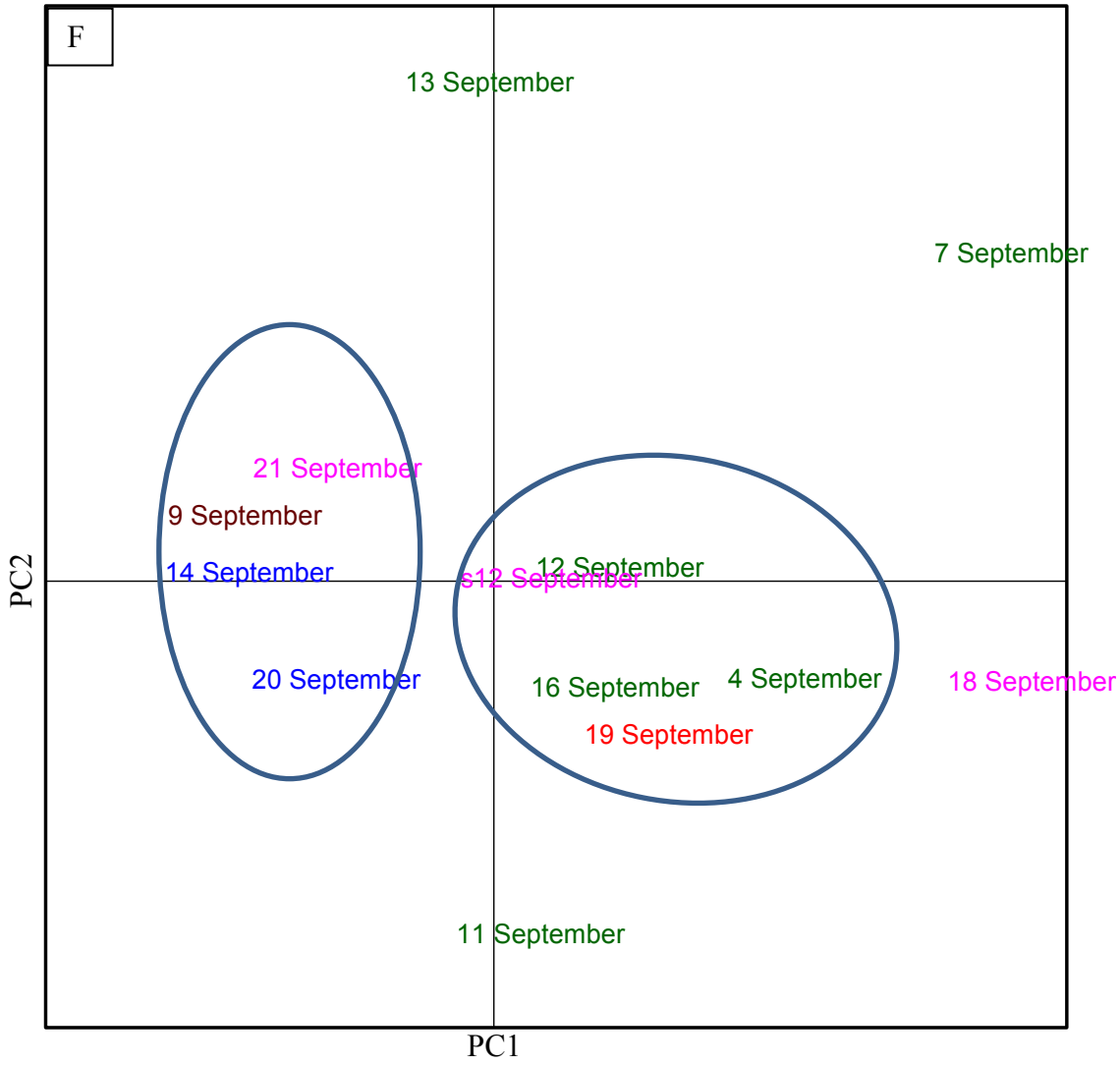


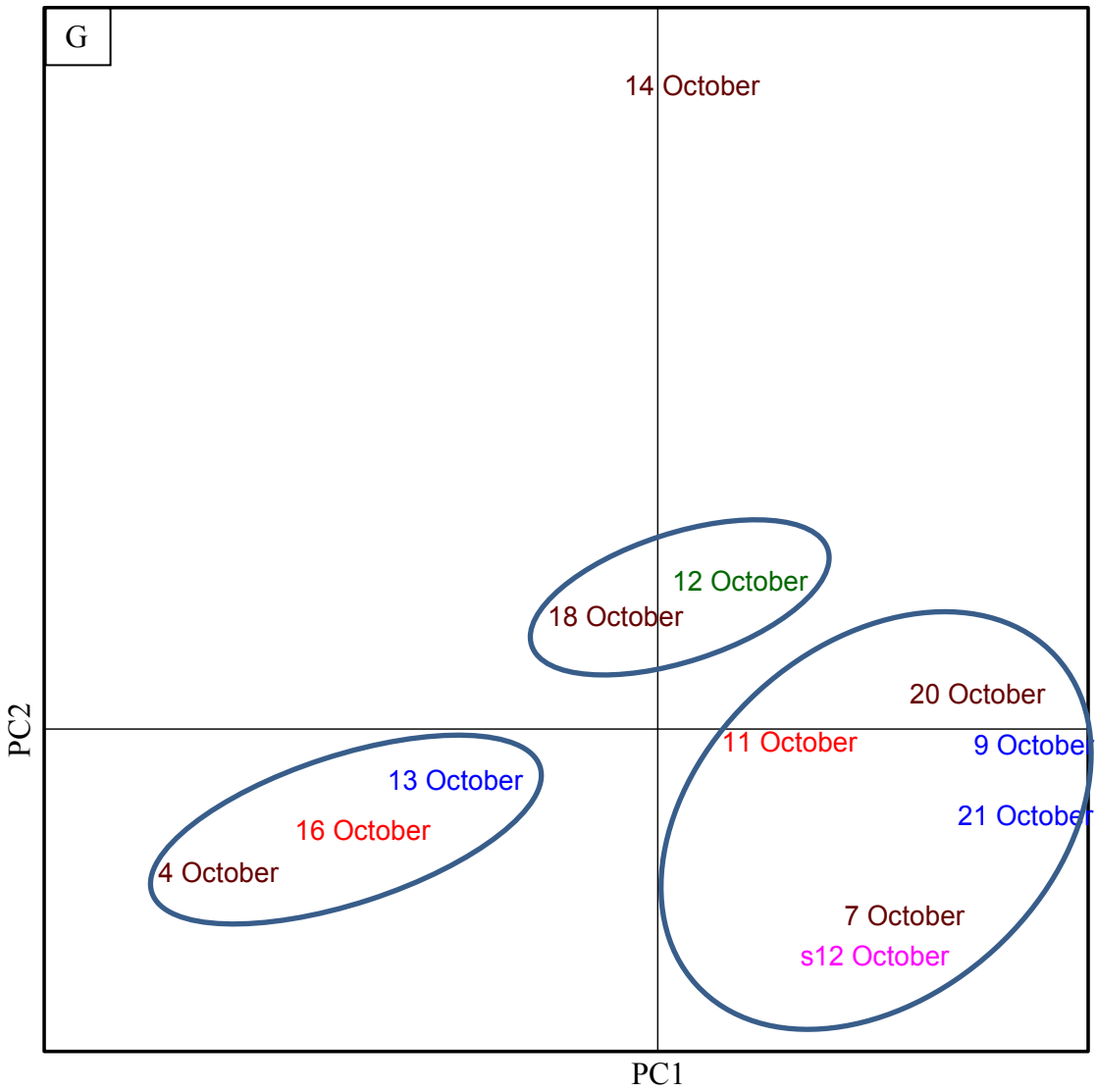

B
21 May
2 May
4 May
20 May
16 May
9 May
7 May
18 May
13 May
19 May
12 May
11 May
14 May
PC2
PC1
Legend:
Dead
0
1
2
3


C
9 June
11 June
12 June
12 June
13 June
18 June
7 June
2 June
19 June
13 June
16 June
21 June
14 June
20 June
4 June
PC2
PC1
Legend:
Dead
0
1
2
3


D
13 July
14 July
16 July
19 July
4 July
7 July
18 July
s3 July
s12 July
21 July
11 July
20 July
12 July
9 July
PC2
PC1
Legend:
Dead
0
1
2
3


E
18 August
21 August
7 August
13 August
20 August
9 August
11 August
14 August
12 August
PC2
PC1
Legend:
Dead
0
1
2
3


F
13 September
7 September
21 September
9 September
14 September
12 September
s12 September
20 September
16 September
4 September
18 September
19 September
11 September
PC2
PC1
Legend:
Dead
0
1
2
3

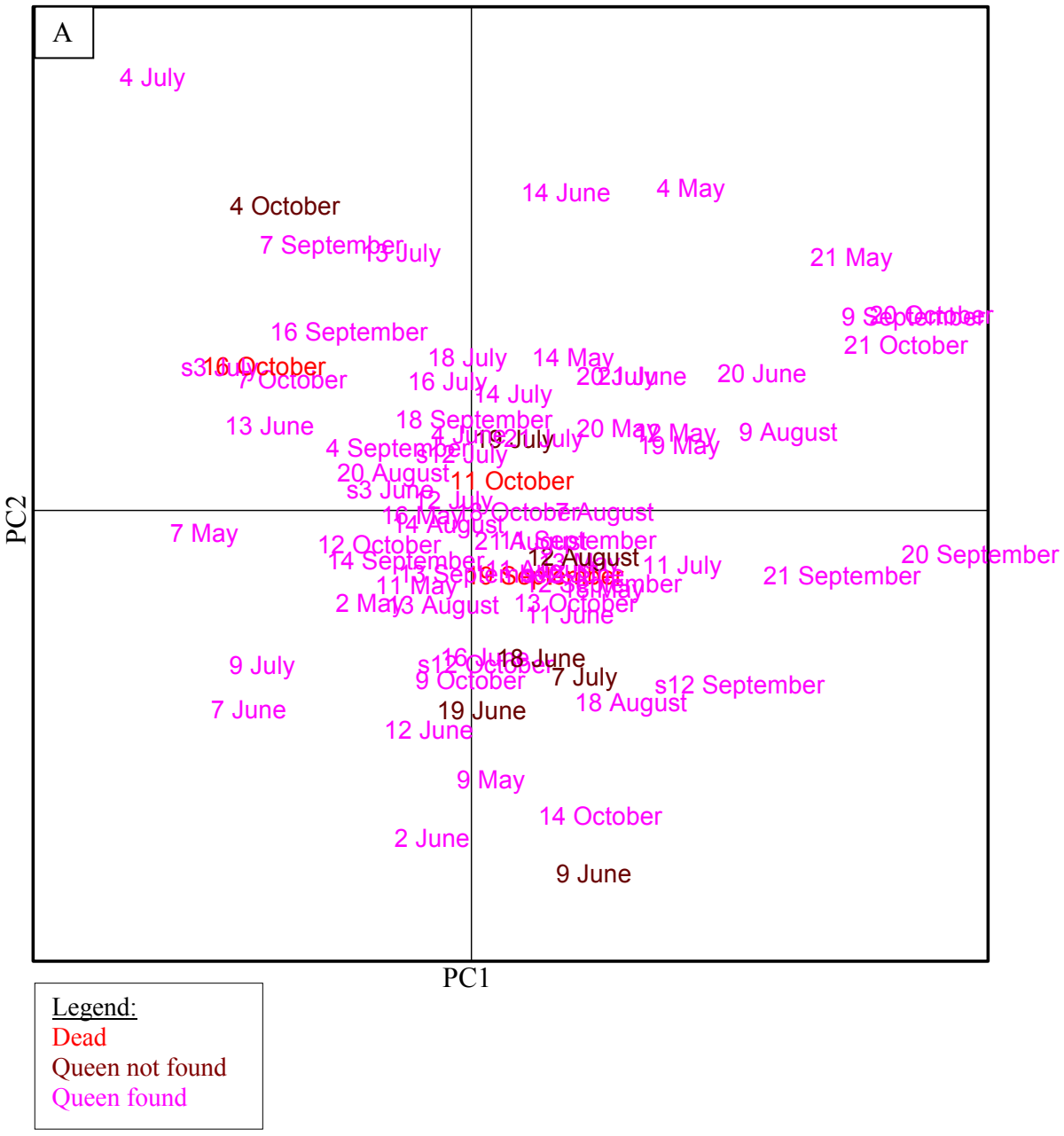


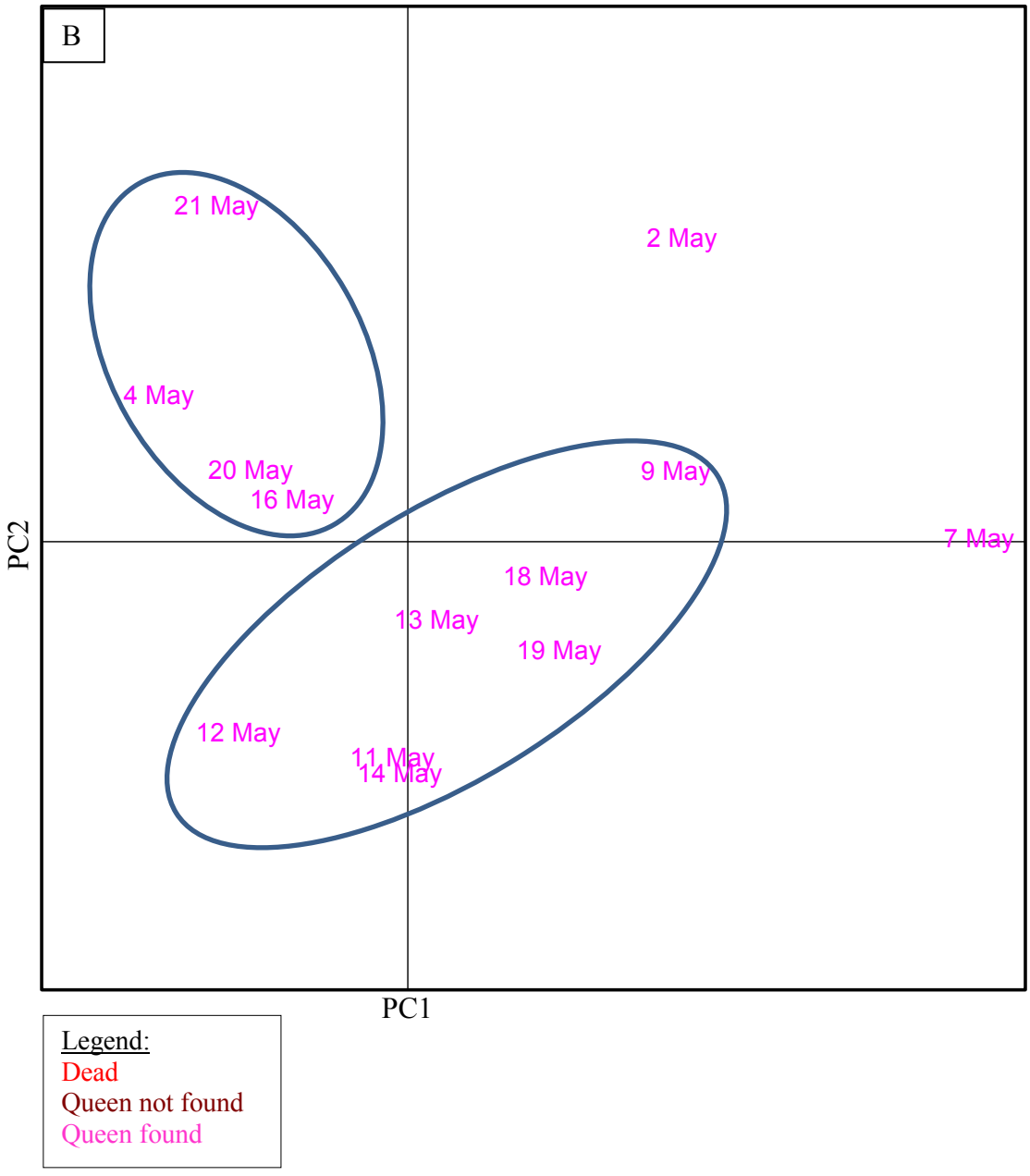
Legend:
Dead
0
1
2
3

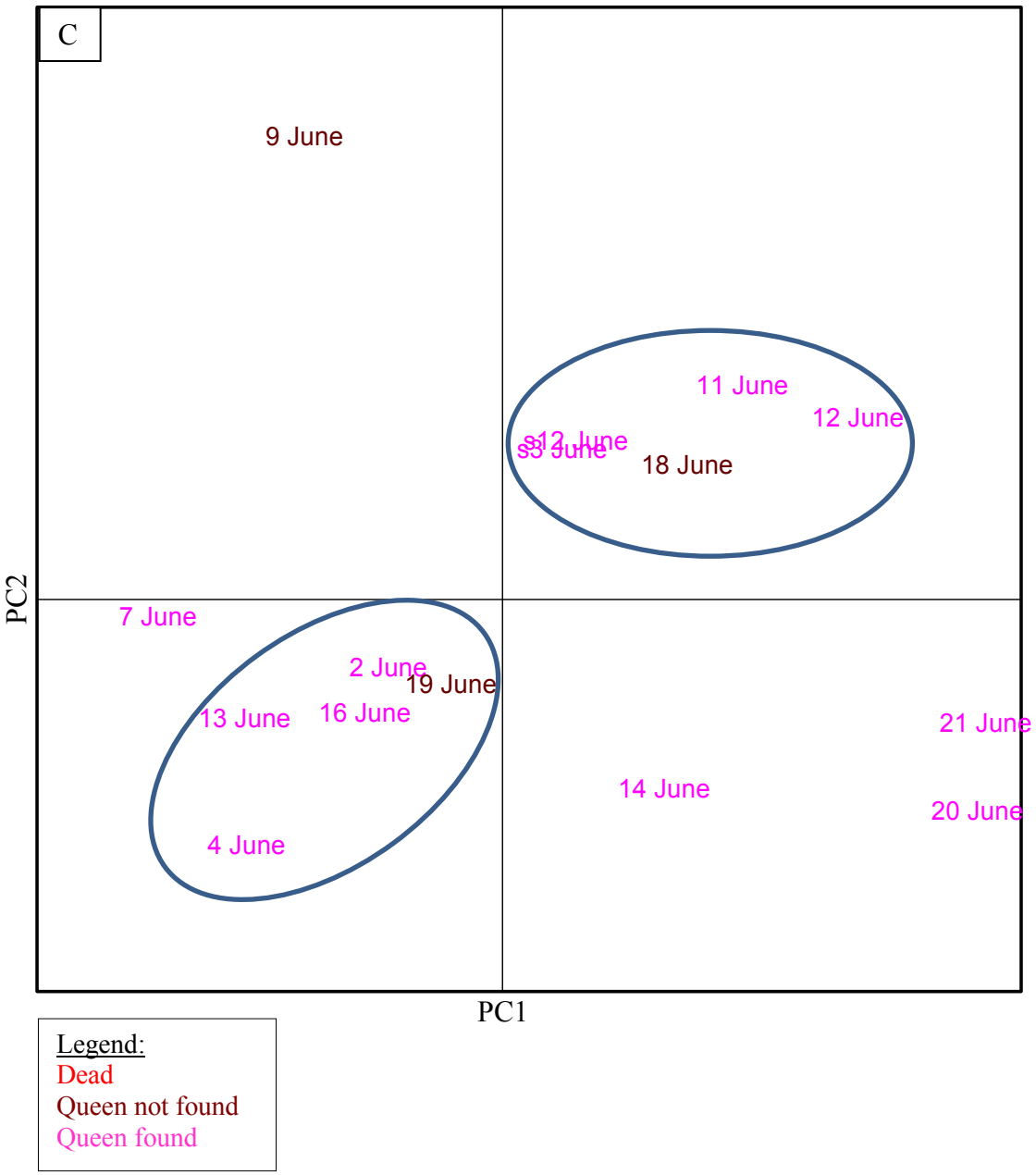
Figure H1 A-G: PCA of all DGGE bacterial profiles of bee bread sampled monthly from the South Campus Research Apiary by the brood pattern. Preceding numbers indicate which colony the bee bread was sampled from and the following month indicates the month the bee bread was sampled. A.) 2D PCA of all bee bread samples. The first and second components represent 5.4% and 5.0% of the variation respectively. B.) 2D PCA of May bee bread samples. The first and second components represent 16.8% and 12.8% of the variation respectively. C.) 2D PCA of June bee bread samples. The first and second components represent 15.2% and 11.3% of the variation respectively. D.) 2D PCA of July bee bread samples. The first and second components represent 14.9% and 12.8% of the variation respectively. E.) 2D PCA of August bee bread samples. The first and second components represent 19.4% and 16.5% of the variation respectively. F.) 2D PCA of September bee bread samples. The first and and second components represents 14.6% and 12.2% of the variation respectively. G.) 2D PCA of October bee bread samples. The first and second components represent 17.3% and 14.9% of the variation respectively.

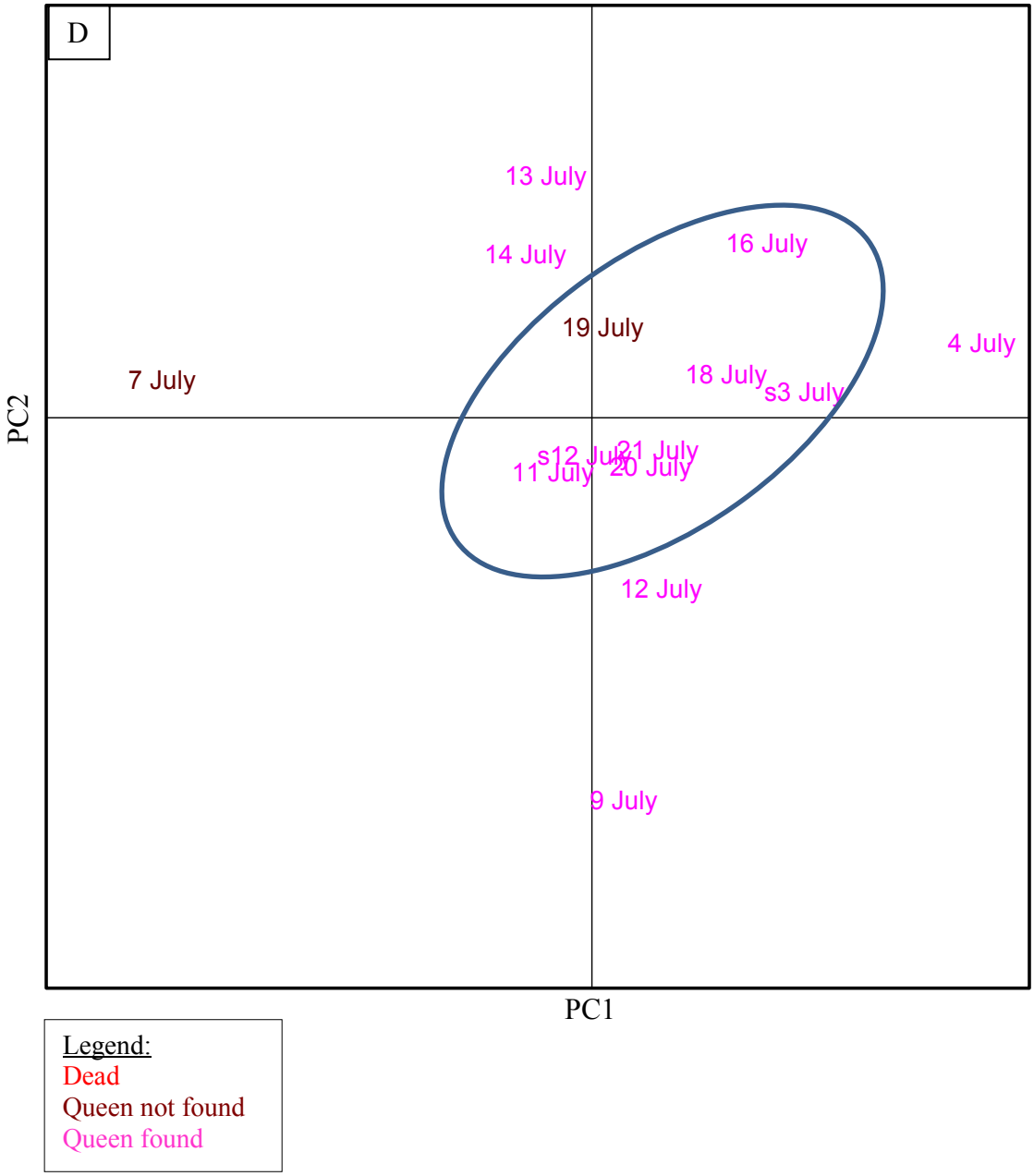
APPENDIX I

PCA OF ALL BEE BREAD BACTERIAL PROFILES BY QUEEN STATUS

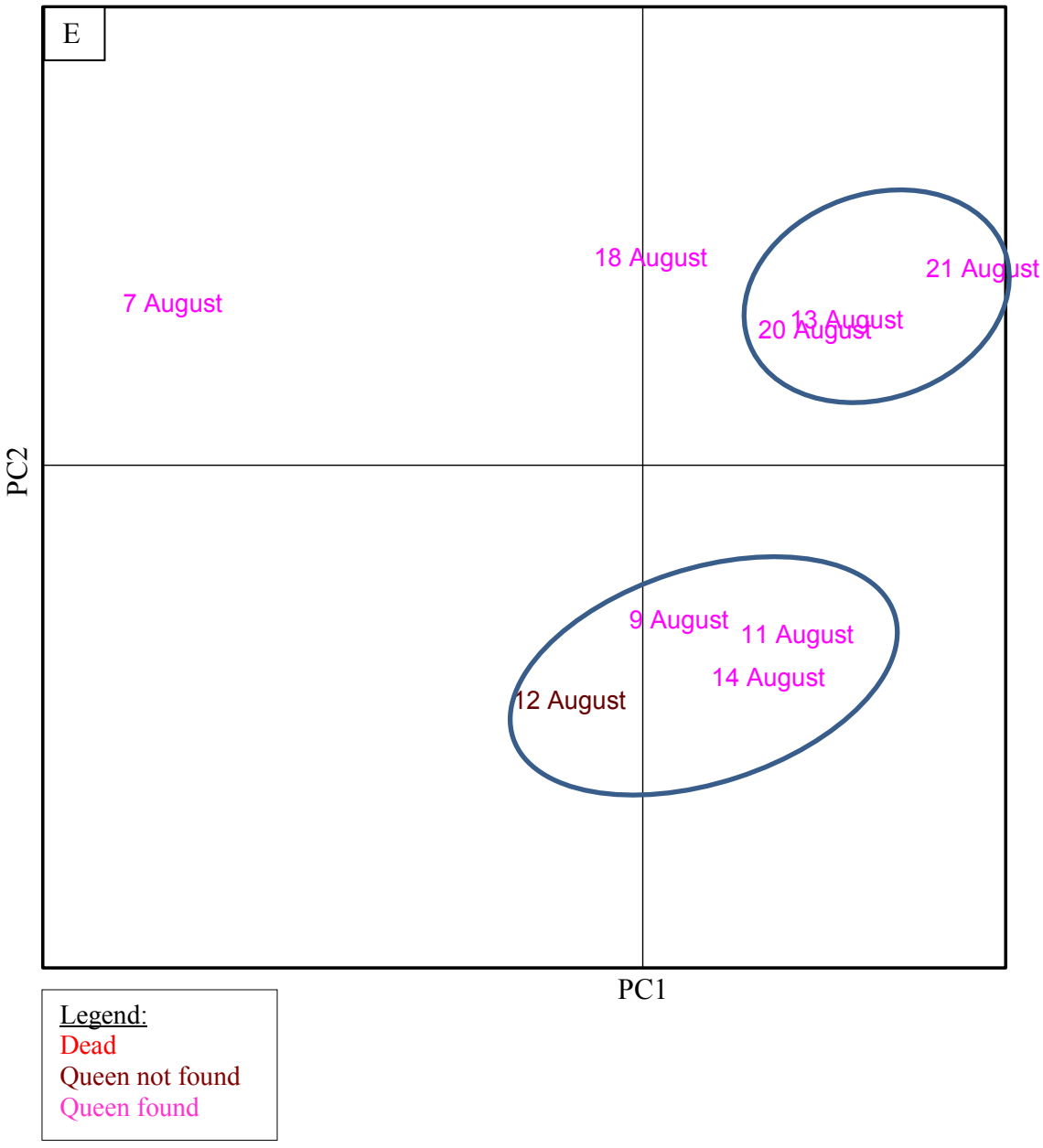


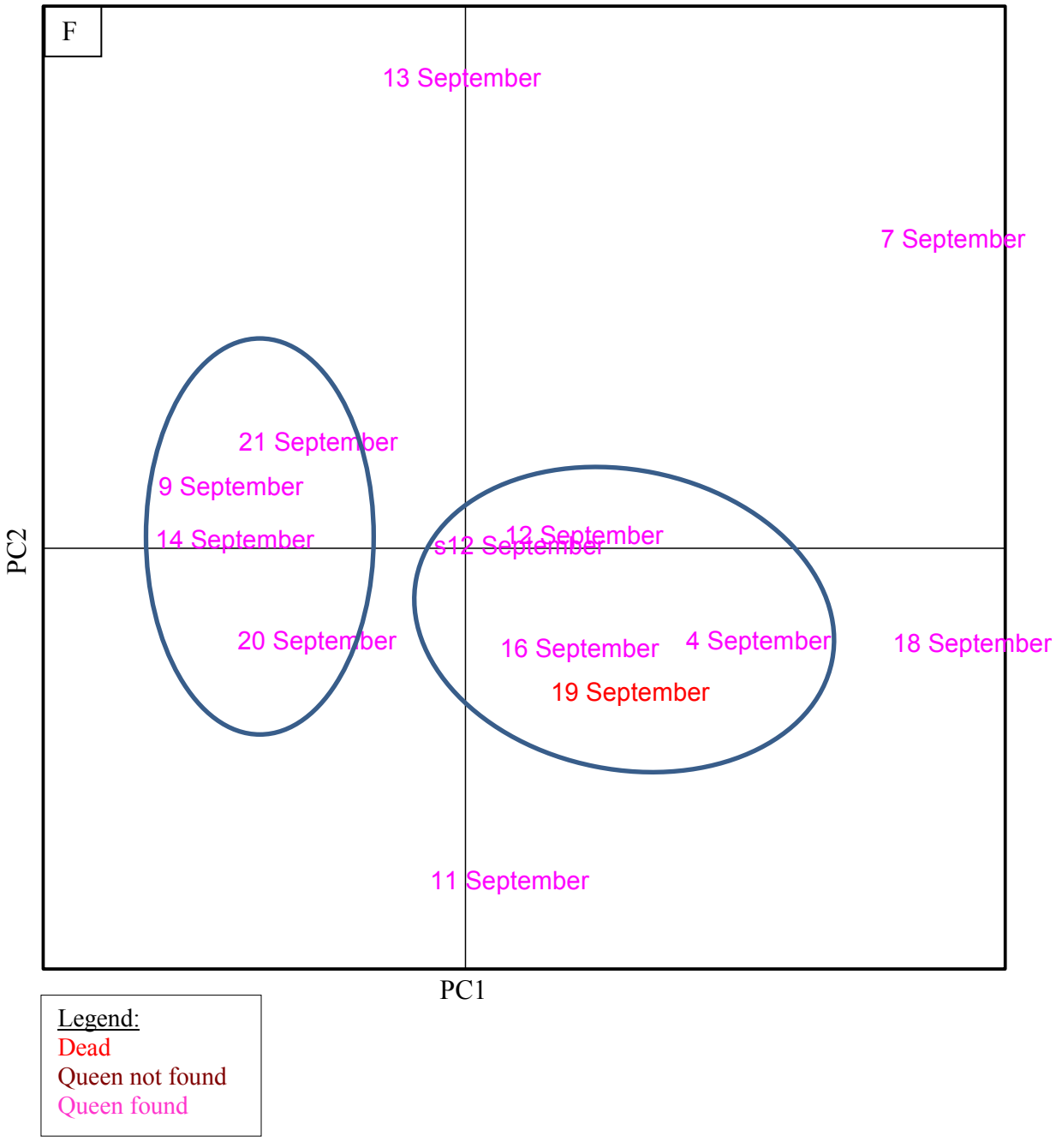

B
21 May
2 May
4 May
20 May
16 May
9 May
7 May
18 May
13 May
19 May
12 May
11 May
14 May
PC2
PC1
Legend:
Dead
Queen not found
Queen found


C
9 June
11 June
12 June
13 June
3 June
18 June
7 June
2 June
19 June
13 June
16 June
21 June
14 June
20 June
4 June
PC2
PC1
Legend:
Dead
Queen not found
Queen found



Legend:
Dead
Queen not found
Queen found


E
18 August
21 August
7 August
13 August
20 August
9 August
11 August
14 August
12 August
PC2
PC1
Legend:
Dead
Queen not found
Queen found


F
13 September
7 September
21 September
9 September
14 September
12 September
s12 September
20 September
16 September
4 September
18 September
19 September
11 September
PC2
PC1
Legend:
Dead
Queen not found
Queen found

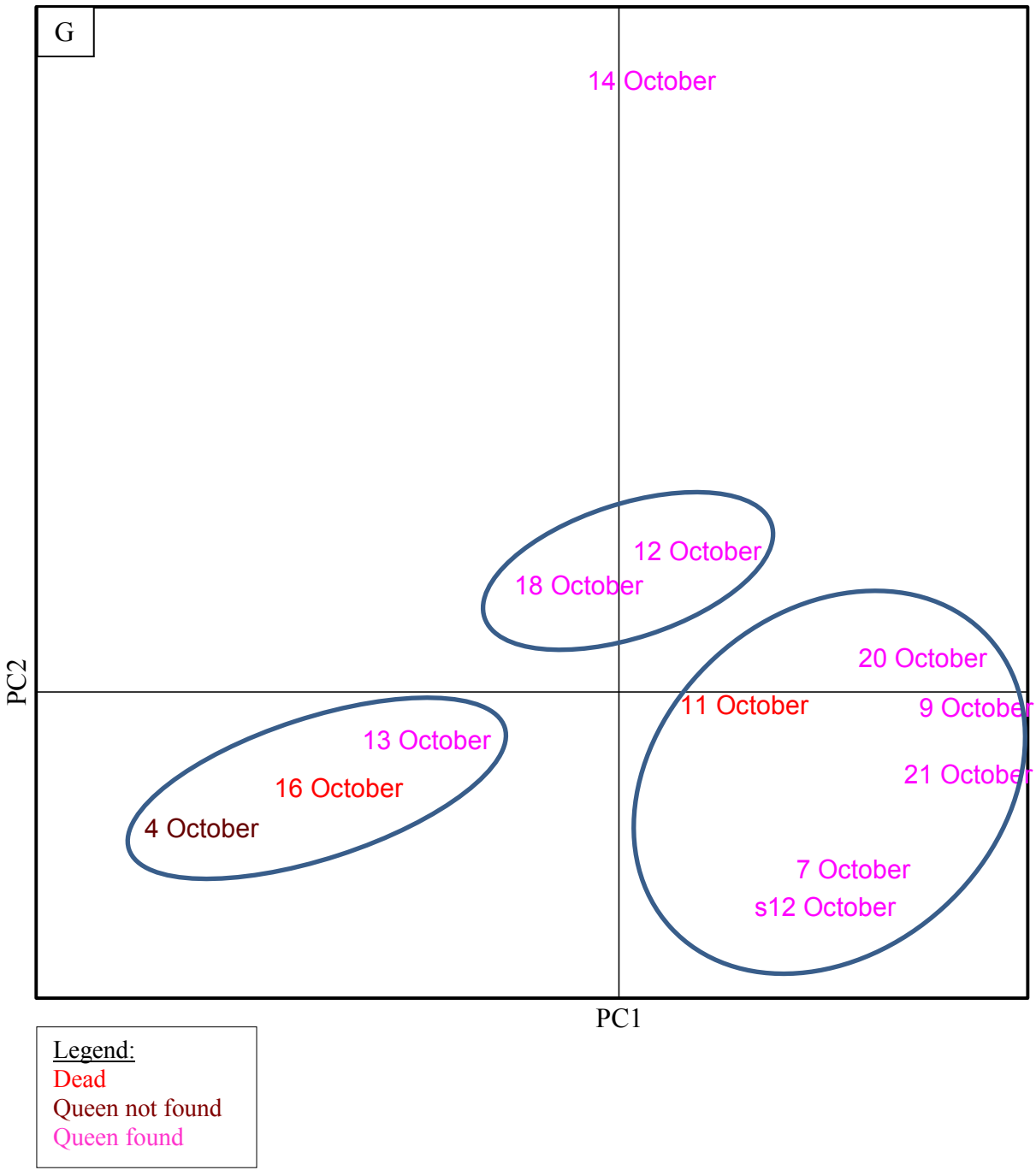
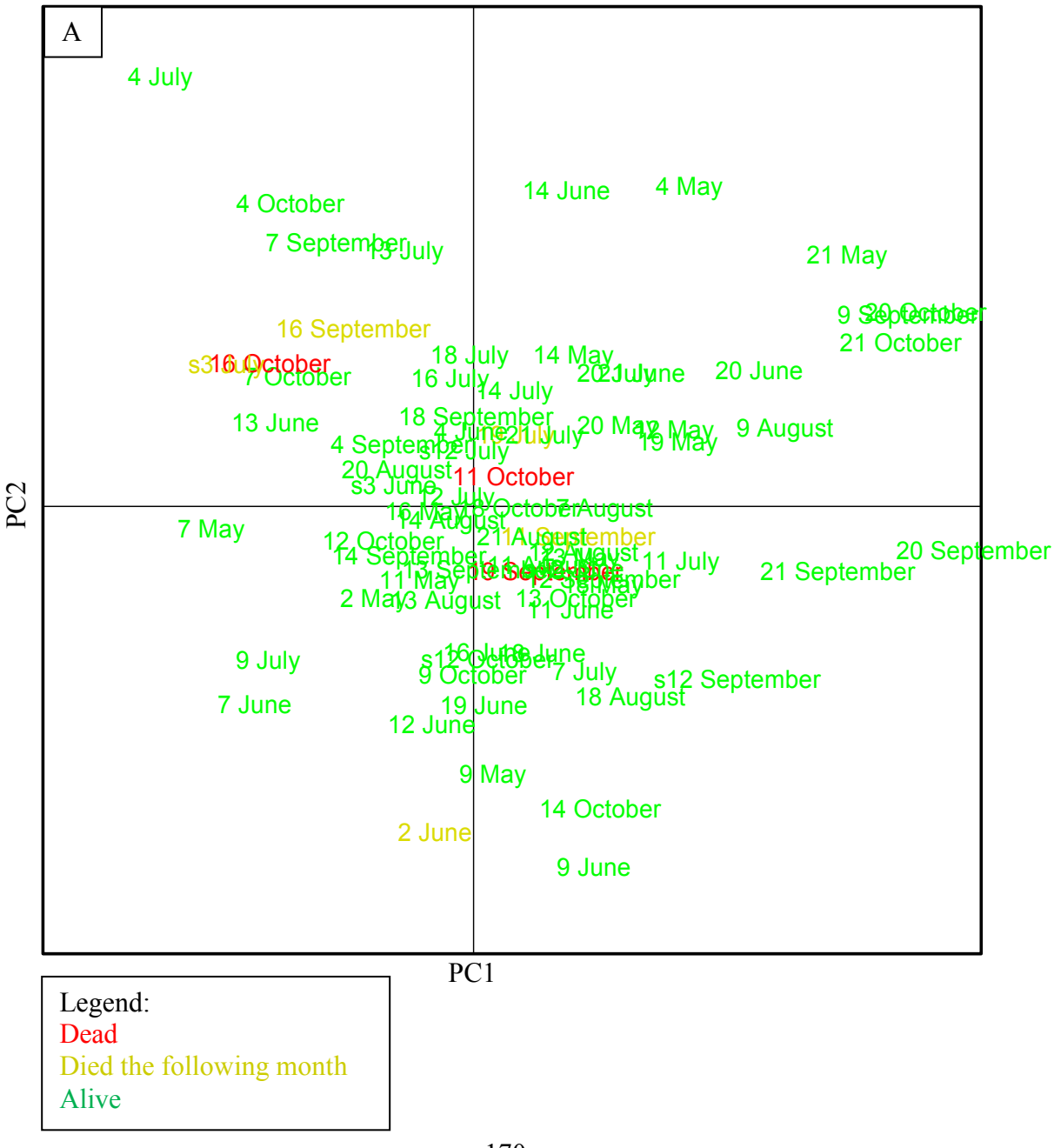
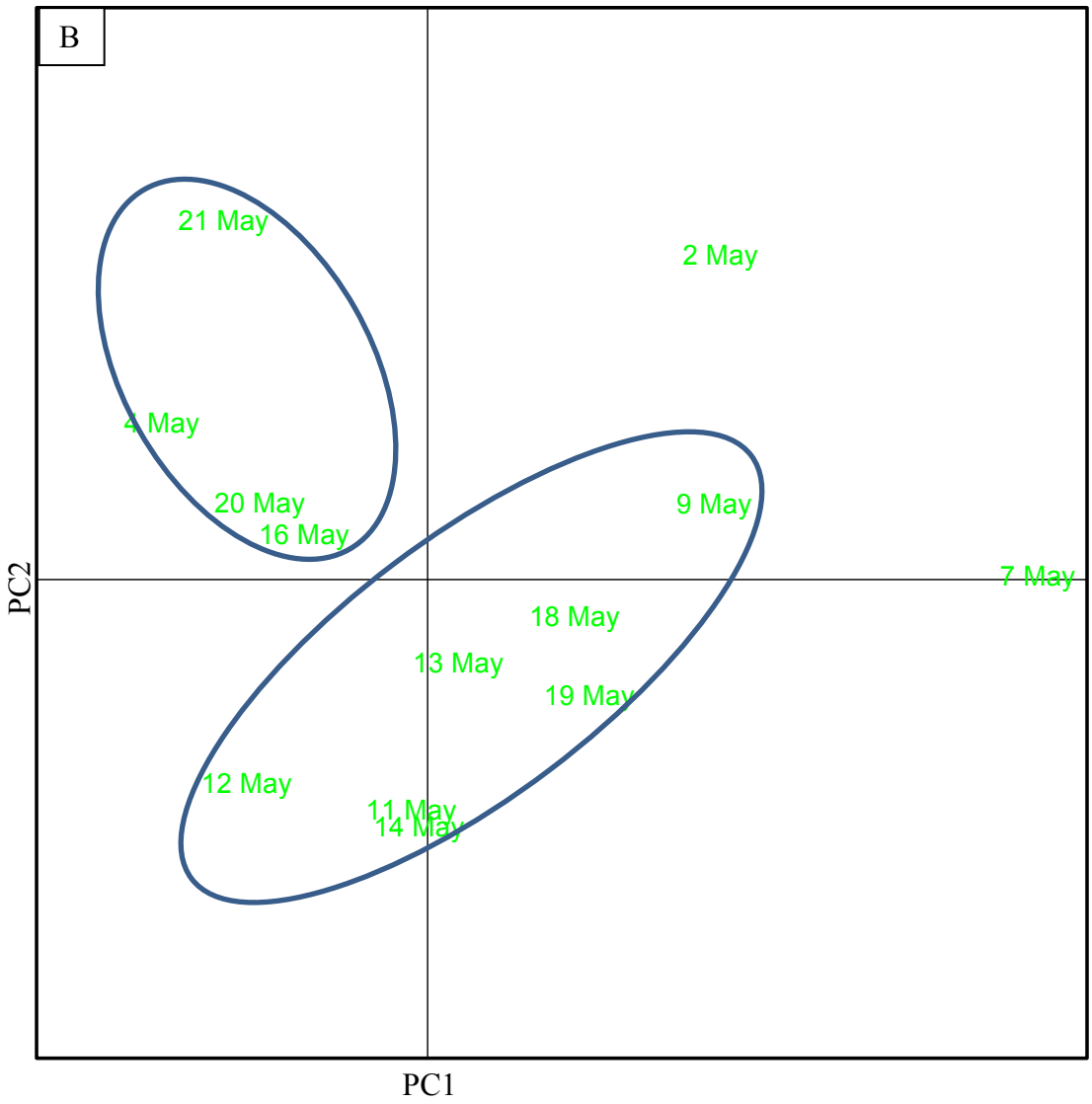

G
14 October
12 October
18 October
20 October
11 October
9 October
13 October
21 October
16 October
4 October
7 October
s12 October
PC2
PC1
Legend:
Dead
Queen not found
Queen found

Figure I1 A-G: PCA of all DGGE bacterial profiles of bee bread sampled monthly from the South Campus Research Apiary by the queen status. Preceding numbers indicate which colony the bee bread was sampled from and the following month indicates the month the bee bread was sampled. A.) 2D PCA of all bee bread samples. The first and second components represent 5.4% and 5.0% of the variation respectively. B.) 2D PCA of May bee bread samples. The first and second components represent 16.8% and 12.8% of the variation respectively. C.) 2D PCA of June bee bread samples. The first and second components represent 15.2% and 11.3% of the variation respectively. D.) 2D PCA of July bee bread samples. The first and second components represent 14.9% and 12.8% of the variation respectively. E.) 2D PCA of August bee bread samples. The first and second components represent 19.4% and 16.5% of the variation respectively. F.) 2D PCA of September bee bread samples. The first and and second components represents 14.6% and 12.2% of the variation respectively. G.) 2D PCA of October bee bread samples. The first and second components represent 17.3% and 14.9% of the variation respectively.

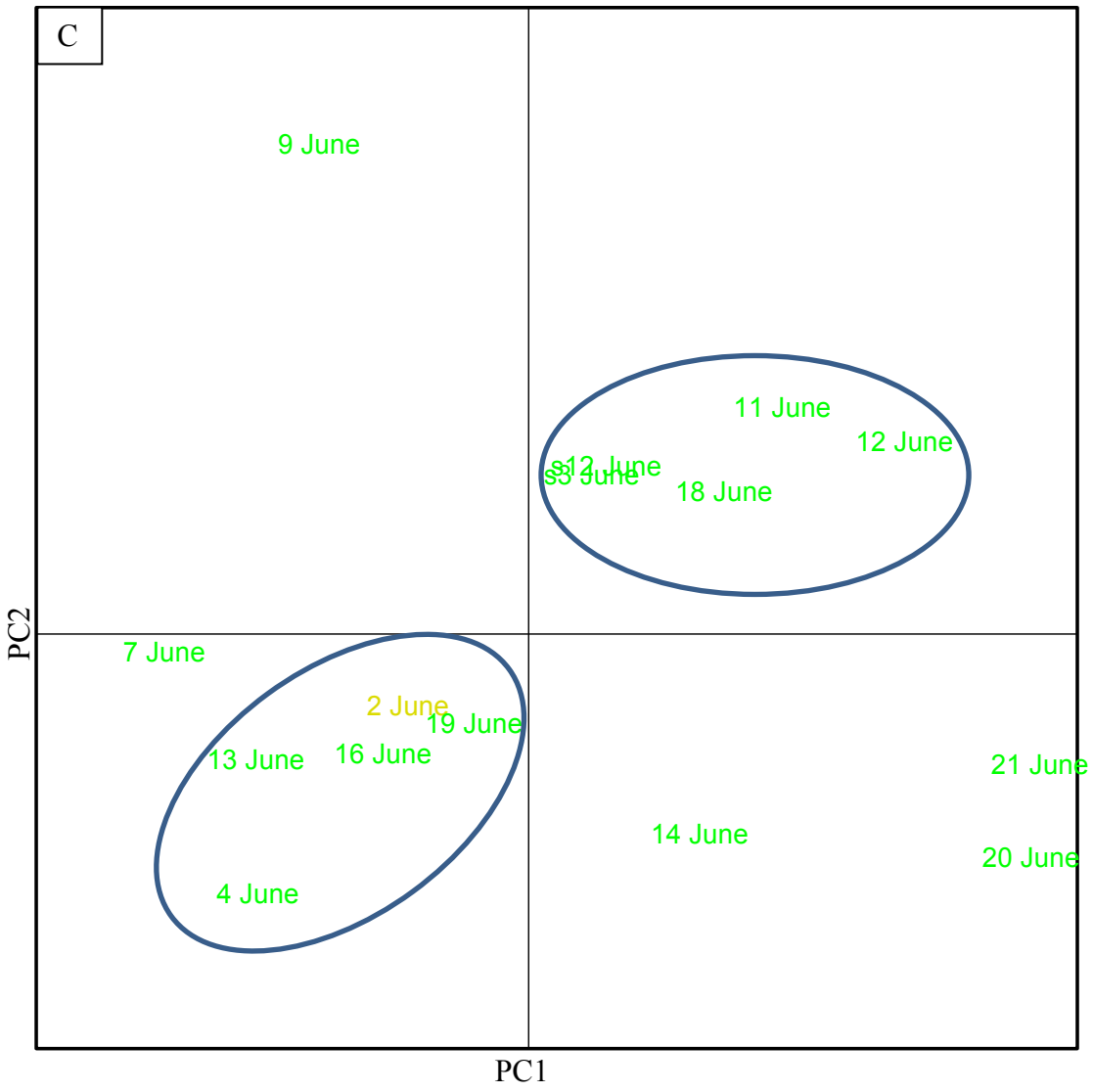
APPENDIX J

PCA OF ALL BEE BREAD BACTERIAL PROFILES BY MONTHLY SURVIVAL STATUS

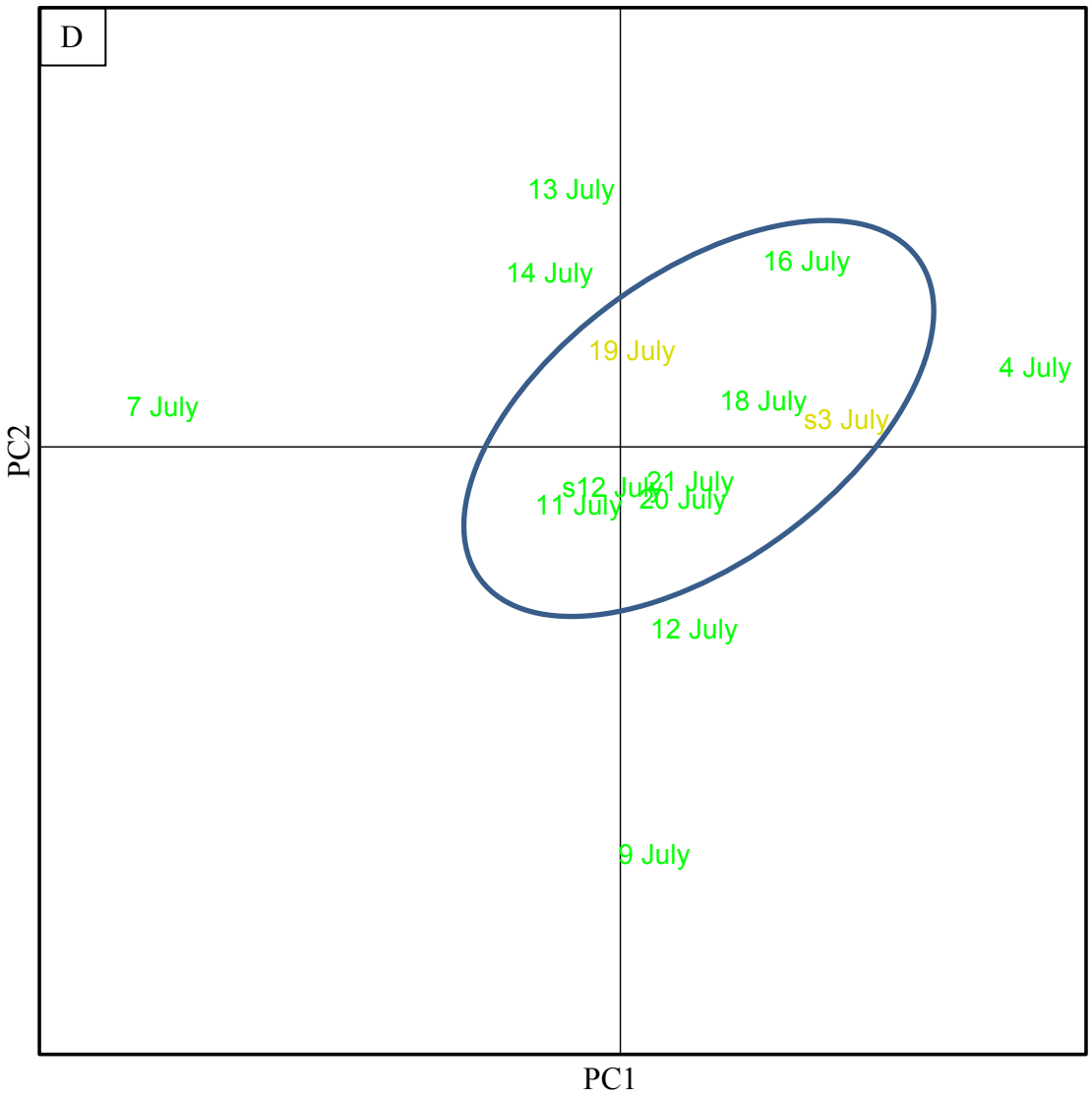




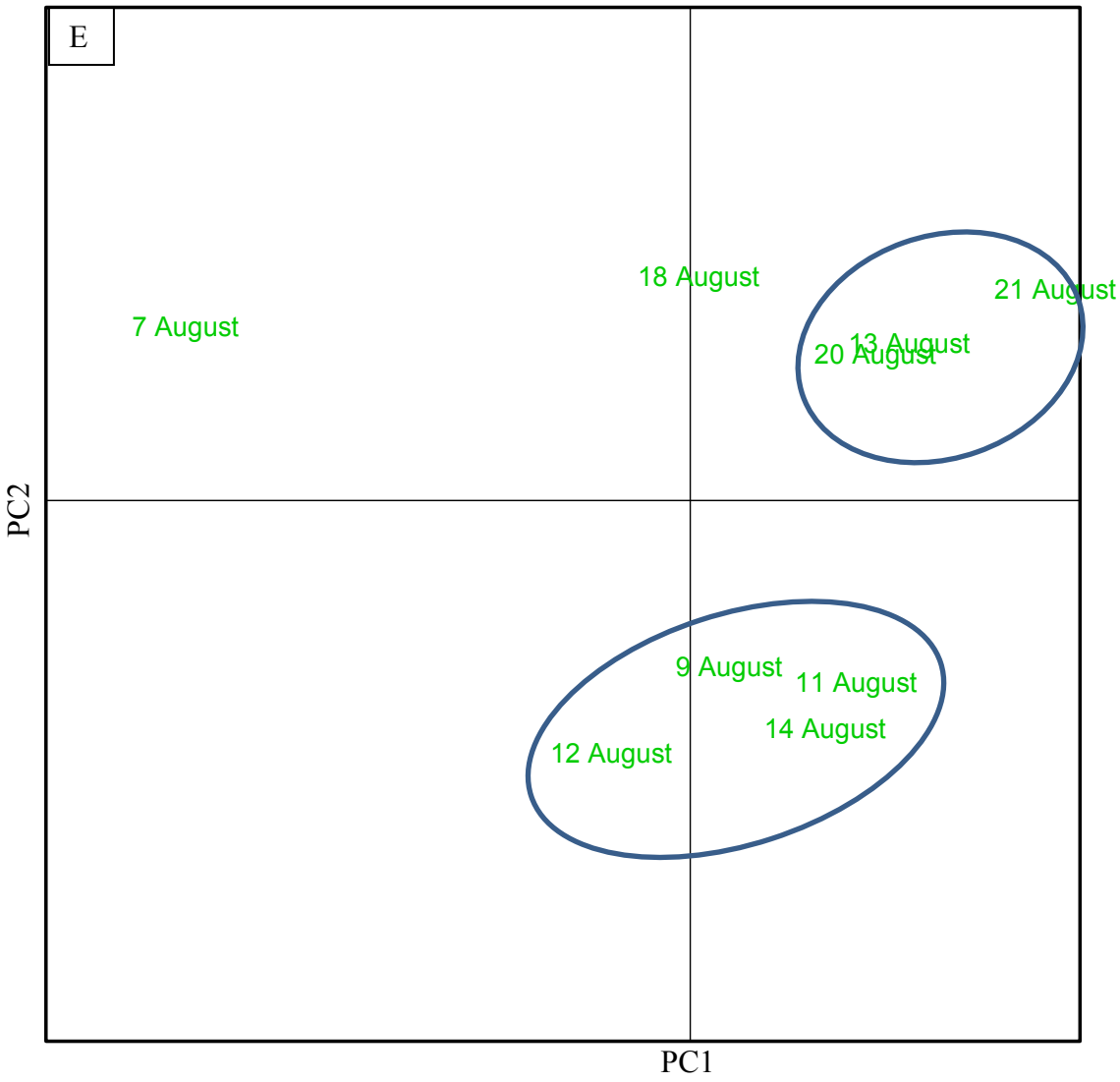
Legend:
Dead
Died the following month
Alive



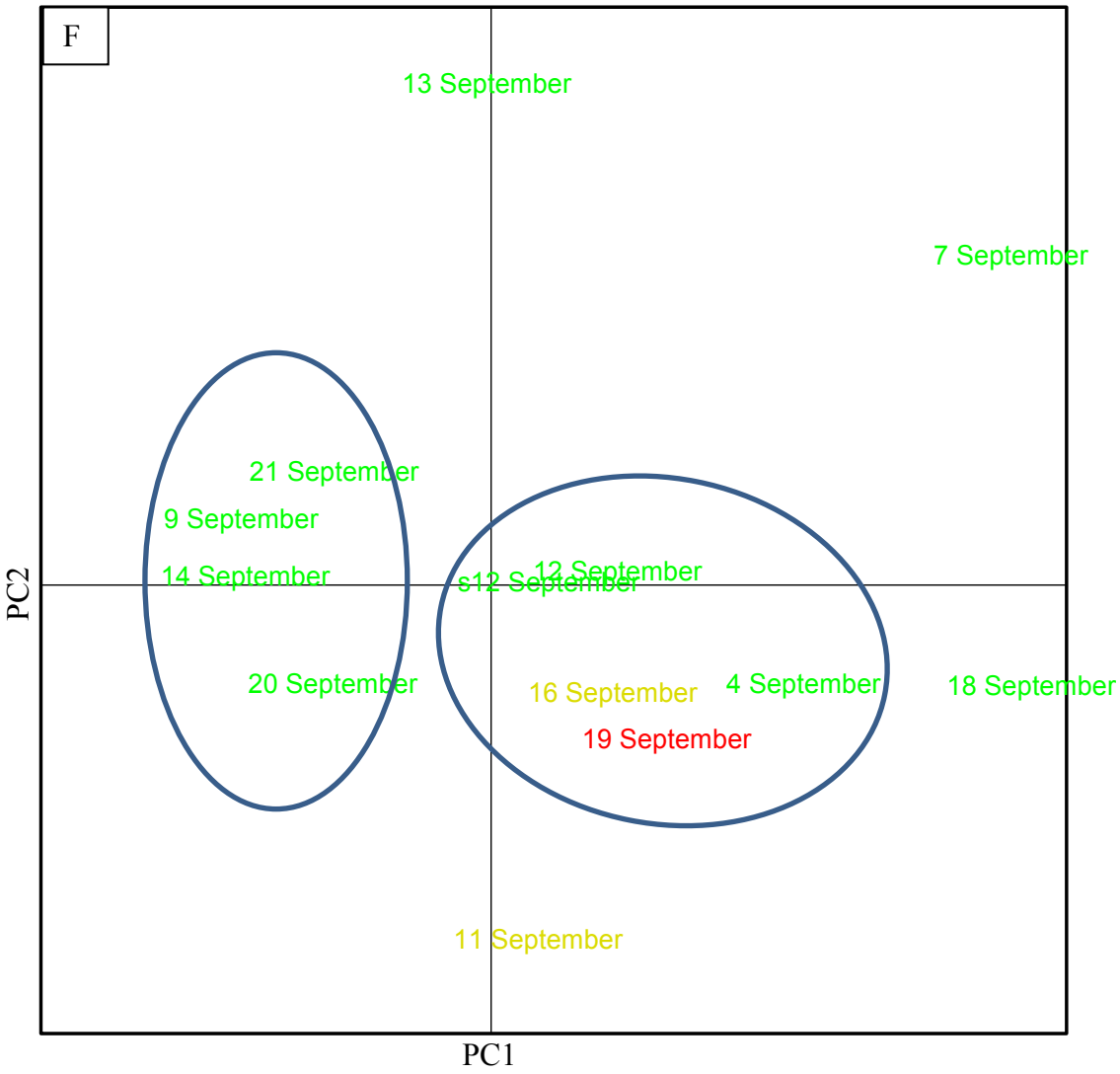
Legend:
Dead
Died the following month
Alive



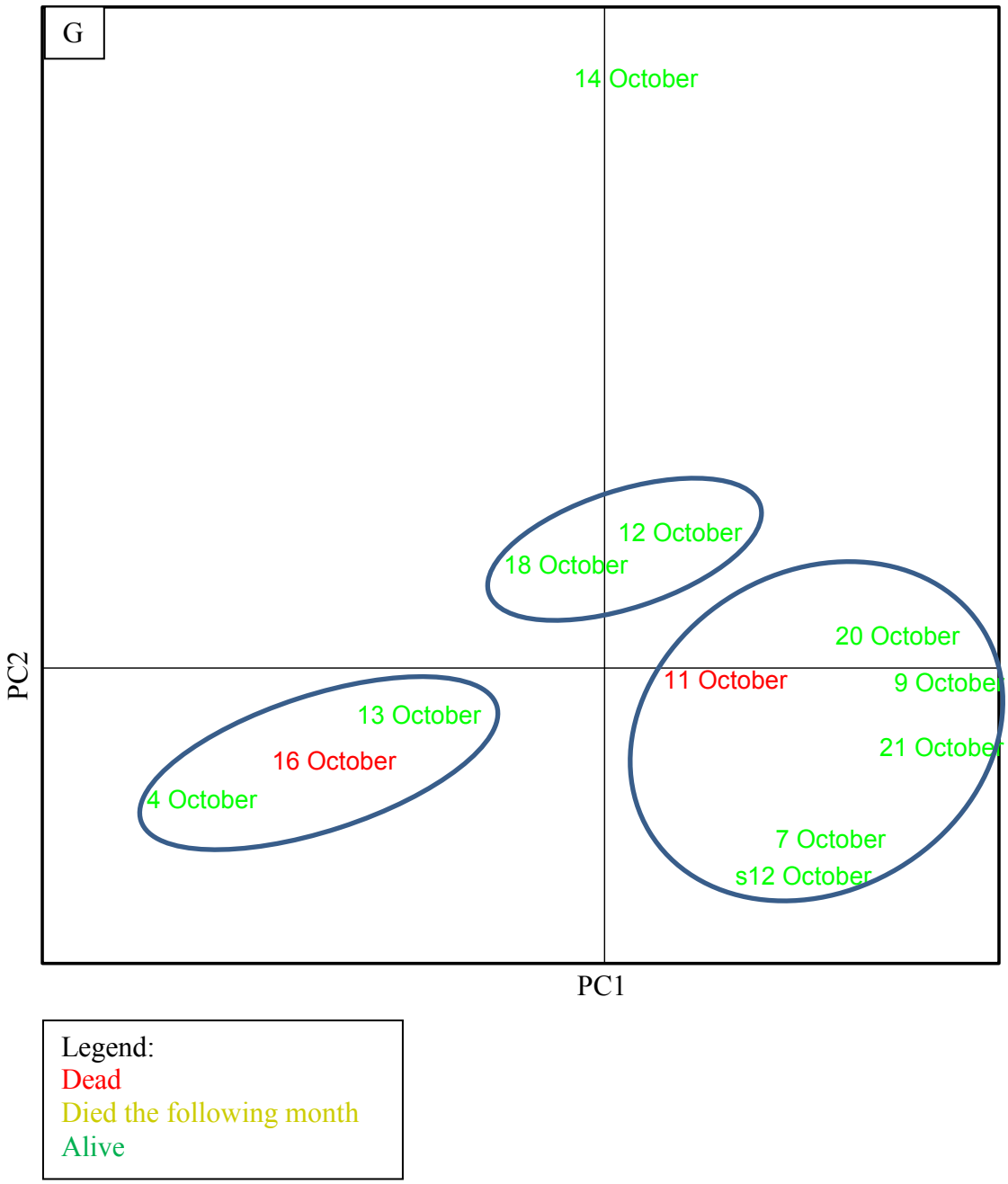
Legend:
Dead
Died the following month
Alive



Legend:
Dead
Died the following month
Alive



Legend:
Dead
Died the following month
Alive



Legend:
Dead
Died the following month
Alive

Figure J1 A-G: PCA of all DGGE microbial profiles of bee bread sampled monthly from the South Campus Research Apiary by the survival of honey bee colonies each month. Preceding numbers indicate which colony the bee bread was sampled from and the following month indicates the month the bee bread was sampled. A.) 2D PCA of all bee bread samples. The first and second components represent 5.4% and 5.0% of the variation respectively. B.) 2D PCA of May bee bread samples. The first and second components represent 16.8% and 12.8% of the variation respectively. C.) 2D PCA of June bee bread samples. The first and second components represent 15.2% and 11.3% of the variation respectively. D.) 2D PCA of July bee bread samples. The first and second components represent 14.9% and 12.8% of the variation respectively. E.) 2D PCA of August bee bread samples. The first and second components represent 19.4% and 16.5% of the variation respectively. F.) 2D PCA of September bee bread samples. The first and and second components represents 14.6% and 12.2% of the variation respectively. G.) 2D PCA of October bee bread samples. The first and second components represent 17.3% and 14.9% of the variation respectively.

APPENDIX K

BACTERIAL IDENTIFICATION BY FAME ANALYSIS RESULTS

Identification of bacteria by FAME analysis. The isolate name indicates the organism's number out of 549 isolates (first number) the month of its sampling (letter) and the colony the bee bread was sampled from (second number). The letter "r" indicates the organism was tested a second time and was chosen to represent the identification of the isolate because the Sim index was higher or had a greater difference from the next closest match in the library. Green rows indicate the species of the organism was identified because the Sim index was above 0.600 and had at least a 0.100 difference from the next name in the library. Purple rows indicate the organism was identified at the species level, but was in the *Enterobacteriaceae* family. Yellow rows indicate only the genus of the organism was identified or it did not have at least a 0.100 difference from the next closest match in the library. White rows indicate the Sim index was below 0.600 and was not identified. Dark red rows indicate no match for the isolated was in the library. Red cells indicate a different organism was identified when it was later tested than when it was originally identified. Grey cells indicate the organism was unable to be tested by FAME analysis.

Isolate Name	Highest Sim Index	Identification	Final Name
71E20	0.530146222	Cedecea-davisae	Enterobacteriaceae
72E20	0.640205017	Salmonella-enterica-enterica E	Salmonella Enterica
73E20	0.688992219	Yersinia-aldovae	Enterobacteriaceae
74-1E20	0.932870149	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
74-2E20	0.543740668	Yersinia-pseudotuberculosis-GC subgroup B	Enterobacteriaceae
75-1E20	0	NO MATCH	no match
75-2E20	0.464600518	Bacillus-cereus-GC subgroup A	Sim index too low
75-3E20	0.511812518	Cedecea-davisae	Enterobacteriaceae
76-1E20	0.769760699	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
76-2E20	0.582637032	Yersinia-bercovieri	Enterobacteriaceae
76-3E20	0.631220901	Cedecea-davisae	Enterobacteriaceae
77-1E20	0.768686012	Bacillus-cereus-GC subgroup A	Bacillus cereus
77-2E20	0.703579845	Bacillus-cereus-GC subgroup A	Bacillus cereus
78-1E20	0.160381157	Pseudomonas-putida-biotype A	Sim index too low
78-2E20r	0.687404456	Bacillus-coagulans	Bacillus coagulans

78-3E20r	0.575389883	Pseudomonas-putida-biotype A	Sim index too low
78-2E20	0.835146129	Bacillus-cereus-GC subgroup A	Bacillus cereus
79E20	0.395176537	Acinetobacter-calcoaceticus	Enterobacteriaceae
80E20	0.915843282	Pantoea-agglomerans-GC subgroup A	Enterobacteriaceae
81-1E20	0.757494411	Pectobacterium-carotovorum-carotovorum	Enterobacteriaceae
81-2E20	0.485163674	Cedecea-davisae	Enterobacteriaceae
82E20	0.524601733	Yersinia-pseudotuberculosis-GC subgroup B	Enterobacteriaceae
83E20	0.360368263	Cedecea-davisae	Enterobacteriaceae
84E20	0.899580467	Pantoea-agglomerans-GC subgroup A	Enterobacteriaceae
85E20	0.444608066	Acinetobacter-calcoaceticus	Sim index too low
87E20	0.493085904	Rahnella-aquatilis	Enterobacteriaceae
88E20	0.967381248	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
88E20r	0.700930491	Microbacterium-barkeri (gram positive)	Microbacterium barkeri
90E20	0.565417077	Cedecea-davisae	Enterobacteriaceae
91E20	0.735847251	Serratia-plymuthica	Serratia plymuthica
92E20	0.79662758	Salmonella-enterica-enterica E	Enterobacteriaceae
93E20	0.721582632	Salmonella-enterica-enterica E	Salmonella enterica
94E10	0.87377569	Bacillus-thuringiensis-israelensis	Bacillus genus
95E20	0.650906844	Salmonella-enterica-enterica E	Salmonella enterica
97E7	0.339595153	Bacillus-megaterium-GC subgroup A	sim index too low
98E7	0.510959617	Bacillus-megaterium-GC subgroup A	sim index too low
99E7	0.48434201	Bacillus-megaterium-GC subgroup A	Sim index too low
100E5	0.73024207	Bacillus-atrophaeus	Bacillus genus
101E5	0.535420415	Bacillus-cereus-GC subgroup A	sim index too low
102E5	0.278859762	Pseudomonas-putida-biotype A	Sim index too low
103E5	0.699929368	Serratia-plymuthica	Serratia plymuthica
104E5	0.68863743	Serratia-plymuthica	Enterobacteriaceae
105E5	0.800490609	Salmonella-enterica-enterica E	Enterobacteriaceae
106E5	0.72230305	Serratia-plymuthica	Enterobacteriaceae
107E5r	0.839396596	Kluyvera-intermedia	Kluyvera intermedia
108E5r	0.856386196	Enterobacter-hormaechei	Enterobacter hormaechei
109E5	0.809756504	Kluyvera-intermedia	Enterobacteriaceae
110E5	0.375745583	Acinetobacter-calcoaceticus	Sim index too low
111-1E5	0.457555822	Chryseobacterium-balustinum	Sim index too low
111-2E5	0.461556939	Chryseobacterium-balustinum	Sim index too low

112E5	0.603973209	Shigella-sonnei-GC subgroup B	Enterobacteriaceae
113E5	0.882751727	Pantoea-agglomerans-GC subgroup B	Pantoea agglomerans
114E5	0.816726141	Kluyvera-intermedia	Enterobacteriaceae
115-1E5	0.775786084	Serratia-plymuthica	Serratia plymuthica
115-2E5	0.743174058	Kluyvera-intermedia	Enterobacteriaceae
116E5	0.599716918	Bacillus-cereus-GC subgroup A	Bacillus cereus
117E5	0.889376113	Pantoea-ananatis/Erwinia uredovora	Pantoea ananatis/Erwinia uredovora
119-4	0.720844648	Bacillus-mycoides-GC subgroup B	Bacillus mycoides
119-1E5	0.574359351	Cedecea-davisae	Enterobacteriaceae
119-2E5	0.835704363	Bacillus-mycoides-GC subgroup B	Bacillus mycoides
119-3E5	0.768093952	Kluyvera-ascorbata-GC subgroup B	Enterobacteriaceae
119-5E5	0.589062237	Cedecea-davisae	Enterobacteriaceae
120-2E5	0.625370405	Bacillus-mycoides-GC subgroup B	Bacillus mycoides
121E5			not tested, did not grow enough
122E5			not tested
123E1	0.476633152	Arthrobacter-aurescens	sim index too low
124E1	0.540638095	Bacillus-licheniformis	Sim index too low
1250 (should be 125E1)	0.550332176	Bacillus-licheniformis	sim index too low
126E1	0.241037653	Brevibacillus-choshinensis	sim index too low
127E5	0	NO MATCH	no match
127-1E1 (should be 127-1E5)	0.347275517	Bacillus-GC group 22	Sim index too low
127-2E1 (Should be 127-2E5)	0.519275751	Bacillus-circulans-GC subgroup A	sim index too low
127-4E1 (Should be 127-4E5)	0.610188513	Bacillus-circulans-GC subgroup B	Bacillus circulans
128E5	0.555671056	Salmonella-enterica-enterica E	Sim index too low
129E5	0.57986687	Dickeya-chrysanthemi-biotype II	Enterobacteriaceae
130-1E5	0.701836486	Rhodococcus-erythropolis/R.globerulus/N.globerula	Rhodococcus erythropolis
131-1E10	0.479032842	Bacillus-megaterium-GC subgroup A	sim index too low
131-2E10	0.330952526	Staphylococcus-hyicus	sim index too low
132E10			not tested
133E16	0.137194038	Serratia-marcescens-GC subgroup C	sim index too low
133E16	0.123003012	Serratia-marcescens-GC subgroup C	sim index too low
133E16r	0.882530027	Bacillus-amyloliquefaciens	Bacillus

			amyloliquefaciens
134E16	0.611852632	Serratia-plymuthica	Enterobacteriaceae
135E16	0.864839107	Bacillus-cereus-GC subgroup A	Bacillus genus
136-1Es12	0.662479548	Staphylococcus-hominis-hominis	Staphylococcus hominis
136-2Es12	0.557467792	Staphylococcus-hominis-hominis	Sim index too low
137Es12	0.938440602	Bacillus-cereus-GC subgroup A	Bacillus genus
138Es12	0.907059477	Bacillus-thuringiensis-israelensis	Bacillus genus
139E18			no 139
140-1E18	0.333915847	Arthrobacter-nicotianae-GC subgroup C	Sim index too low
140-1E18	0.356003968	Arthrobacter-nicotianae-GC subgroup C	Sim index too low
140-1E18c	0	NO MATCH	no match
141E18			not tested
142-1E18	0.605117053	Bacillus-thuringiensis-canadensis	Bacillus genus
142-2E18	0.586293286	Bacillus-megaterium-GC subgroup A	sim index too low
143E18	0.565713831	Bacillus-megaterium-GC subgroup A	sim index too low
144E18	0.780889469	Bacillus-cereus-GC subgroup A	Bacillus cereus
145E18	0.812161311	Bacillus-cereus-GC subgroup A	Bacillus cereus
146Es3	0.877854611	Bacillus-cereus-GC subgroup A	Bacillus cereus
147E11			not tested
148E11	0.782019328	Bacillus-cereus-GC subgroup A	Bacillus genus
149E11	0.507092889	Salmonella-enterica-enterica E	Enterobacteriaceae
150E11	0.108076311	Pseudomonas-putida-biotype A	sim index too low
151E11			not tested
152E11	0.708246452	Escherichia-coli-GC subgroup G	Enterobacteriaceae
153E11	0.800234666	Bacillus-cereus-GC subgroup A	Bacillus cereus
154E11	0.796703027	Escherichia-coli-GC subgroup C	Enterobacteriaceae
155-1E11	0.751890849	Cedecea-davisae	Enterobacteriaceae
155-2E11	0.390843232	Bacillus-megaterium-GC subgroup A	sim index too low
156E11	0.767490743	Kluyvera-cryocrescens-GC subgroup B	Enterobacteriaceae
158-1E11	0.839445373	Enterobacter-asburiae	Enterobacter genus
158-2E11	0.659908264	Cedecea-davisae	Enterobacteriaceae
159			no 159
160E5	0.788529114	Acetobacter-pasteurianus	Acetobacter pasteurians
161-1E11	0.509013618	Bacillus-mycoides-GC subgroup A	sim index too low
161-2E11	0.764682503	Bacillus-mycoides-GC subgroup A	Bacillus mycoides

162E11			not tested
163E11	0.561721912	Yersinia-frederiksenii	Enterobacteriaceae
164E11	0.736356625	Salmonella-enterica-enterica E	Salmonella enterica
165E11	0.716285004	Cedecea-davisae	Enterobacteriaceae
166Es12	0.78328035	Bacillus-cereus-GC subgroup A	Bacillus cereus
167-1E11	0.908656432	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
167-2E11	0.865490135	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
168Y3	0.674864735	Staphylococcus-xylosus-GC subgroup B	Staphylococcus xylosus
169			no 169
170Y3	0.716402767	Serratia-liquefaciens	Enterobacteriaceae
171-1Y3	0.570798441	Bacillus-pumilus-GC subgroup B	sim index too low
171-2Y3	0.625765039	Yersinia-bercovieri	Enterobacteriaceae
172Y3	0.773257826	Citrobacter-freundii	Enterobacteriaceae
173Y3	0.791334049	Sphingomonas-parapaucimobilis	Sphingomonas genus
174Y3	0.149080178	Enterococcus-avium	sim index too low
175Y3	0.311236604	Grimontia-hollisae	sim index too low
176Y3	0.723518176	Proteus-vulgaris	Proteus vulgaris
177Ys3	0.842697424	Bacillus-cereus-GC subgroup A	Bacillus cereus
178Ys3	0.889000264	Bacillus-thuringiensis-canadensis	Bacillus genus
179Y4	0.79655115	Kluyvera-intermedia	Enterobacteriaceae
180-1Y4	0.74308022	Proteus-vulgaris	Enterobacteriaceae
180-2Y4	0.830573046	Staphylococcus-xylosus-GC subgroup B	Staphylococcus xylosus
181Y4	0.635802439	Salmonella-enterica-enterica E	Salmonella enterica
182Y4	0.796622333	Proteus-vulgaris	Enterobacteriaceae
183Y4	0.878276323	Rhodococcus-erythropolis/R.globerulus/N.globerula	Rhodococcus erythropolis
184Y4	0.731562162	Proteus-vulgaris	Enterobacteriaceae
185Y4	0	NO MATCH	no match
186Y4	0.653815514	Yersinia-bercovieri	Yersinia bercovieri
187Y4	0.778202504	Proteus-vulgaris	Proteus vulgaris
188Y7	0.643707104	Bacillus-pumilus-GC subgroup B	Bacillus pumilus
189Y7	0.784312199	Paenibacillus-polymyxa	Paenibacillus polymyxa
190Y7	0.842677087	Bacillus-cereus-GC subgroup A	Bacillus cereus
191Y7	0.237104291	Bacillus-cereus-GC subgroup A	sim index too low
192Y9	0.715631788	Bacillus-thuringiensis-canadensis	Bacillus genus

193Y9			not tested
194Y9	0.683922902	Bacillus-cereus-GC subgroup A	Bacillus cereus
195Y9	0.74953239	Paenibacillus-polymyxa	Paenibacillus polymyxa
196Y9	0.514146016	Arthrobacter-nicotianae-GC subgroup C	sim index too low
197Y9	0.280713699	Bacillus-cereus-GC subgroup A	sim index too low
198Y9	0.58054343	Bacillus-cereus-GC subgroup A	sim index too low
199Y9	0.738717113	Bacillus-cereus-GC subgroup A	Bacillus genus
200Y10	0.86992661	Bacillus-subtilis	Bacillus subtilis
201Y10	0.734132839	Bacillus-cereus-GC subgroup B	Bacillus cereus
202Y10	0.471544118	Bacillus-megaterium-GC subgroup A	sim index too low
203Y10	0.918048259	Bacillus-subtilis	Bacillus subtilis
204Y10	0.957701002	Bacillus-subtilis	Bacillus subtilis
205Y11	0.608197181	Bacillus-mycoides-GC subgroup B	Bacillus mycoides
206Y11	0.897094258	Bacillus-thuringiensis-canadensis	Bacillus thuringiensis-canadensis
207Y12	0	NO MATCH	no match
208Y12	0.935909594	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
209Y12	0.640503024	Bacillus-mycoides-GC subgroup B	Bacillus mycoides
210Y12	0	NO MATCH	no match
211Y12	0.431626544	Arthrobacter-nicotianae-GC subgroup C	sim index too low
212Y12	0.922257093	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
213Y12	0.667673223	Bacillus-sphaericus-GC subgroup C	Bacillus sphaericus
214Y12	0.691408497	Arthrobacter-nicotianae-GC subgroup C	Arthrobacter nicotianae
215Y12			not tested
216Y19	0.516619716	Bacillus-megaterium-GC subgroup A	sim index too low
217Y19	0.900896283	Bacillus-cereus-GC subgroup A	Bacillus cereus
218Y19			not tested
219E19	0.821247601	Bacillus-sphaericus-GC subgroup C	Bacillus sphaericus
220-2Y19	0.928955001	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
221-1M2	0.794804966	Bacillus-cereus-GC subgroup A	Bacillus genus
221-2M2	0.845667383	Micrococcus-luteus-GC subgroup B	Micrococcus luteus
222M2	0.70033589	Micrococcus-luteus-GC subgroup B	Micrococcus luteus
223M2	0.596372577	Bacillus-pumilus-GC subgroup B	sim index too low
224M2	0.4766009	Bacillus-megaterium-GC subgroup A	sim index too low

225-1M2	0.897357834	Brevibacillus-reuszeri	Brevibacillus reuszeri
225-1M2	0.765525907	Micrococcus-luteus-GC subgroup B	Micrococcus luteus
226M2c	0.319373812	Bacillus-circulans-GC subgroup B	sim index too low
227M3	0.55667839	Virgibacillus-pantothenticus	sim index too low
228M3	0.529559745	Bacillus-cereus-GC subgroup A	sim index too low
229M3	0.516167816	Virgibacillus-pantothenticus	sim index too low
230M3	0.532725604	Bacillus-thuringiensis-canadensis	sim index too low
231M3	0.649060921	Bacillus-megaterium-GC subgroup B	Bacillus genus
232M3	0.47748844	Bacillus-pumilus-GC subgroup A	sim index too low
233M3	0.579899089	Virgibacillus-pantothenticus	sim index too low
234M3	0.921272055	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
235M4	0.854985923	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
236M4	0.553955243	Bacillus-GC group 22	sim index too low
237M4	0.709897122	Micrococcus-lylae-GC subgroup A	Micrococcus genus
238M3	0.149138379	Listeria-grayi	sim index too low
238M4r	0.285386355	Paenibacillus-azotofixans	not original isolated
239M7	0.590304877	Bacillus-cereus-GC subgroup A	sim index too low
240M7	0.593853258	Bacillus-megaterium-GC subgroup A	sim index too low
241M7	0.635010609	Bacillus-clausii	Bacillus clausii or Hyphomonas hirschiana
242M9	0.433027057	Bacillus-megaterium-GC subgroup A	sim index too low
243M9	0.393642969	Streptoverticillium-reticulum	sim index too low
243M9r	0.148173296	Paenibacillus-macerans	not original isolated
244M9	0.838023535	Bacillus-cereus-GC subgroup A	Bacillus cereus
245M10	0.417058718	Bacillus-pumilus-GC subgroup B	sim index too low
246M10			not tested
247M10	0.424941197	Bacillus-megaterium-GC subgroup A	sim index too low
248M10	0.726187781	Bacillus-cereus-GC subgroup A	Bacillus cereus
249M10	0.679633736	Bacillus-cereus-GC subgroup A	Bacillus genus
250M10			not tested
251M16	0.378927592	Bacillus-pumilus-GC subgroup B	sim index too low
252M16	0.578096225	Bacillus-mycoides-GC subgroup B	sim index too low
253M16	0.887558699	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
254M16	0.882740817	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
255M16	0.71400357	Bacillus-mycoides-GC subgroup B	Bacillus mycoides
256M12	0	NO MATCH	no match
257M12	0.911166873	Brevibacillus-reuszeri	Brevibacillus

			reuszeri
258-1M12	0	NO MATCH	no match
258-2M12	0	NO MATCH	no match
259M11	0.523599553	Bacillus-pumilus-GC subgroup B	sim index too low
260M11	0.624334428	Bacillus-cereus-GC subgroup A	Bacillus cereus
261M11	0	NO MATCH	no match
262M11	0	NO MATCH	no
262M11c	0.240777284	Paenibacillus-pabuli	sim index too low
262M11c	0	NO MATCH	no mtach
263M11	0.901238232	Bacillus-subtilis	Bacillus subtilis
264M11	0.60298504	Bacillus-cereus-GC subgroup A	Bacillus genus
265M11			not tested
267M12	0	NO MATCH	no match
268			no 168
269M19	0.32800285	Bacillus-sphaericus-GC subgroup B	sim index too low
270M19	0.75081822	Bacillus-thuringiensis-canadensis	Bacillus genus
271M19	0.62268373	Bacillus-megaterium-GC subgroup A	Bacillus megaterium or Brevabacillus parabrevis
272M19	0.671492139	Arthrobacter-nicotianae-GC subgroup C	Arthrobacter nicotianae
273M20	0.192914076	Bacillus-GC group 22	sim index too low
274M21			not tested
275M20	0.47629576	Bacillus-megaterium-GC subgroup A	sim index too low
276M21	0.646147121	Bacillus-cereus-GC subgroup A	Bacillus cereus
277		numbers accidentally skipped	no 227
278		numbers accidentally skipped	no 228
279		numbers accidentally skipped	no 229
280M10	0.848179695	Bacillus-cereus-GC subgroup A	Bacillus cereus
281A3	0.956806393	Flavimonas-oryzihabitans	Flavimonas oryzihabitans or Chryseomonas luteola
282A3	0.607952545	Pseudomonas-savastanoi-nerium	Pseudomonas genus
282A3	0.68905727	Staphylococcus-xylosus-GC subgroup B	Staphylococcus xylosus
284A3	0.754098014	Staphylococcus-xylosus-GC subgroup B	Staphylococcus xylosus
285A3	0.704421583	Kocuria-kristinae-GC subgroup A	Kocuria kristinae

285A3	0.476187268	Kocuria-kristinae-GC subgroup A	sim index too low
285A3d	0.710788913	Kocuria-kristinae-GC subgroup B	Kocuria kristinae
286-1A3	0.698372441	Microbacterium-chocolatum	Microbacterium chocolatum
286-2A3	0.700183178	Microbacterium-chocolatum	Microbacterium chocolatum
287A3	0.8806837	Pseudomonas-aeruginosa-GC subgroup A	Pseudomonas aeruginosa
287-1A3	0.687828681	Pseudomonas-aeruginosa-GC subgroup A	Pseudomonas aeruginosa
287-2A3	0	NO MATCH	no match
288A3	0.728751674	Bacillus-thuringiensis-dendrolimus	Bacillus genus
289A3	0	NO MATCH	no match
289-1A3	0.829189647	Microbacterium-barkeri	Microbacterium barkeri
289-2A3	0	NO MATCH	no match
290A3	0.464187491	Sphingobium-yanoikuyae	sim index too low
291-1A3	0.408581708	Sphingobium-yanoikuyae	sim index too low
291-2A3	0.458098077	Sphingomonas-capsulata	sim index too low
292A3			not testd
293A3	0.694751683	Staphylococcus-xylosus-GC subgroup B	Staphylococcus xylosus
294A3	0.761497919	Enterococcus-faecalis-GC subgroup B	Enterococcus faecalis
295A3	0.538563804	Microbacterium-barkeri	sim index too low
296A3	0.795307681	Microbacterium-barkeri	Microbacterium barkeri
297A3	0.526122248	Microbacterium-barkeri	sim index too low
299A3	0.183927661	Cellulomonas-fimi-GC subgroup B	sim index too low
300A3			no tested
301-1A3	0.474060247	Novosphingobium-subterraneum	sim index too low
301-2A3	0.422132201	Novosphingobium-subterraneum	sim index too low
302A3	0.251881091	Streptoverticillium-reticulum	sim index too low
302A3a			not tested
303A3	0.820574892	Staphylococcus-xylosus-GC subgroup B	Staphylococcus xylosus
304A3	0.902557949	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
305A3	0.716260478	Staphylococcus-xylosus-GC subgroup B	Staphylococcus xylosus
306A3	0.477180774	Staphylococcus-xylosus-GC subgroup	sim index too low

		B	
306A3	0.503446555	Paenibacillus-polymyxa	sim index too low
307A3	0.692848147	Staphylococcus-xylosus-GC subgroup B	Staphylococcus xylosus
308A3	0.624464045	Microbacterium-arborescens	Microbacterium arborescens
309A3	0.516546902	Kocuria-kristinae-GC subgroup B	sim index too low
309A3d	0.506340901	Kocuria-kristinae-GC subgroup B	sim index too low
310A3	0.424055067	Novosphingobium-subterraneum	sim index too low
311A3	0.137115249	Novosphingobium-subterraneum	sim index too low
312AS3	0	NO MATCH	no match
313As3	0.63652312	Bacillus-thuringiensis-canadensis	Bacillus thuringiensis-canadensis
314As3	0.817100547	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
315AS3	0	NO MATCH	no match
316A4	0	NO MATCH	no match
317			not tested
318A7	0.654641014	Bacillus-pumilus-GC subgroup B	Bacillus pumilus
318A7d	0.643019182	Bacillus-pumilus-GC subgroup B	Bacillus pumilus
319A7	0.843477463	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
320A7	0.351359735	Bacillus-sphaericus-GC subgroup F	sim index too low
321-1A7	0.57656108	Paenibacillus-polymyxa	sim index too low
322			no 322
323			no 323
324A7	0.486161884	Bacillus-mycoides-GC subgroup B	sim index too low
325-1A7	0.489219188	Bacillus-mycoides-GC subgroup B	sim index too low
325-2A7	0.587018673	Bacillus-megaterium-GC subgroup A	sim index too low
326A7	0.866389644	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
327A7	0.870749313	Bacillus-cereus-GC subgroup A	Bacillus cereus
328A9	0	NO MATCH	no match
329A7	0.572011146	Brevibacillus-reuszeri	sim index too low
330A9	0	NO MATCH	no match
331A10	0.846732099	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
332A10	0.72527186	Bacillus-cereus-GC subgroup A	Bacillus cereus
333			no 333
334			no 334
335-1A10	0.855681367	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
335-2A10	0.81171734	Serratia-plymuthica	Enterobacteriaceae

336A10	0	NO MATCH	no match
337-1A10	0.666076231	Bacillus-cereus-GC subgroup A	Bacillus cereus
337-2A10	0.810508034	Bacillus-cereus-GC subgroup A	Bacillus cereus
338A10	0.767317738	Bacillus-cereus-GC subgroup A	Bacillus genus
339A10	0.526532536	Bacillus-pumilus-GC subgroup B	sim index too low
340A10	0.215346117	Bacillus-GC group 22	sim index too low
341A10	0.562028266	Bacillus-pumilus-GC subgroup B	sim index too low
342A10	0.831477272	Bacillus-thuringiensis-canadensis	Bacillus genus
343A11NO			not tested
344A11	0.947234093	Microbacterium-barkeri	Microbacterium barkeri
345A11	0	NO MATCH	no match
346A11	0.417816333	Microbacterium-chocolatum	sim index too low
347A11	0.835906853	Microbacterium-barkeri	Microbacterium barkeri
348A12	0.407280092	Sphingomonas-capsulata	sim index too low
349A12	0.770939266	Ewingella-americana	Ewingella americana
350A12	0.83883869	Bacillus-thuringiensis-canadensis	Bacillus cereus
351A12	0.824986328	Microbacterium-barkeri	Microbacterium barkeri
352A12	0.952032023	Microbacterium-barkeri	Microbacterium barkeri
353A12	0.734376879	Sphingomonas-sanguinis	Sphingomonas genus
354A12	0.742240092	Cedecea-davisae	Cedecea davisae
354A12r	0.741893767	Cedecea-davisae	Cedecea davisae
355S12			no 355
356As12	0.151293188	Bacillus-mycoides-GC subgroup B	sim index too low
357As12	0.54817663	Bacillus-pumilus-GC subgroup B	Bacillus pumilus
358As12	0.37021079	Bacillus-megaterium-GC subgroup A	sim index too low
359As12	0.882307114	Bacillus-cereus-GC subgroup A	Bacillus cereus
360			
361A13	0	NO MATCH	no match
361A13r	0	NO MATCH	no match
362A13	0.509571448	Bacillus-megaterium-GC subgroup A	sim index too low
362A13r	0.292525336	Bacillus-alcalophilus	sim index too low
363			
364			
365			

366A13	0.54060084	Microbacterium-chocolatum	sim index too low
367A13	0.927207782	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
369A14	0.918555073	Bacillus-cereus-GC subgroup A	Bacillus genus
370A16	0.658895648	Bacillus-cereus-GC subgroup A	Bacillus cereus
371A16	0.484191725	Kurthia-gibsonii	sim index too low
372A16	0.656785682	Bacillus-cereus-GC subgroup A	Bacillus cereus
373A16	0.834630344	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
374A16	0.439482789	Bacillus-megaterium-GC subgroup A	sim index too low
375A16	0.751388058	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
376A16	0.456506446	Bacillus-megaterium-GC subgroup A	sim index too low
377A16	0.549046358	Bacillus-thuringiensis-israelensis	sim index too low
378A16	0.492135143	Bacillus-megaterium-GC subgroup A	sim index too low
379A16	0.10251976	Corynebacterium-diphtheriae-intermedius	sim index too low
378A16			not tested
381A16	0.610239921	Bacillus-cereus-GC subgroup A	Bacillus genus
382A16	0.622684276	Bacillus-marisflavi	Bacillus marisflavi
382-1A16			not tested
382-2A16	0.330525597	Bacillus-mycoides-GC subgroup B	sim index too low
383A18	0.692110661	Bacillus-mycoides-GC subgroup B	Bacillus mycoides
384A18	0.446418403	Kurthia-gibsonii	sim index too low
385A18	0.981651577	Bacillus-subtilis	Bacillus subtilis
386A18			not tested
387A18	0.81927329	Flavimonas-oryzihabitans	Flavimonas oryzihabitans or Pseudomonas aeruginosa
388-1A18	0.845938536	Flavimonas-oryzihabitans	Flavimonas oryzihabitans or Pseudomonas aeruginosa
388-2A18	0.940144255	Bacillus-thuringiensis-israelensis	Bacillus thuringiensis
389-1A18	0.92098772	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
389-1A18r			not tested
389-1A18r			not tested
CR389-1A18r			not tested
390A19	0.882200042	Micrococcus-luteus-GC subgroup B	Micrococcus luteus
391A19			not tested

392A19	0.747027237	Bacillus-cereus-GC subgroup A	Bacillus cereus
393A19	0.71621665	Bacillus-cereus-GC subgroup A	Bacillus genus
394A19	0.822376153	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
395A19			not tested
396A20	0	NO MATCH	no match
369A20c	0.241004989	Bacillus-cereus-GC subgroup A	sim index too low
396A20c	0.236648269	Bacillus-cereus-GC subgroup A	sim index too low
397A20	0.732119335	Bacillus-cereus-GC subgroup A	Bacillus cereus
398A20	0.657444463	Yersinia-aldovae	Yersinia aldove
399A20	0.65036042	Bacillus-pumilus-GC subgroup B	Bacillus pumilus
400A20	0.53311015	Bacillus-mycoides-GC subgroup B	sim index too low
401A20	0.527810117	Bacillus-megaterium-GC subgroup A	sim index too low
402A20	0.77890298	Bacillus-mycoides-GC subgroup B	Bacillus mycoides
403A20	0.824550809	Flavimonas-oryzihabitans	Flavimonas oryzihabitans or Chryseomonas luteola
404A20	0.81112639	Flavimonas-oryzihabitans	Flavimonas oryzihabitans or Chryseomonas luteola
405A20	0.819668815	Flavimonas-oryzihabitans	Flavimonas oryzihabitans or Chryseomonas luteola
406A20	0.895336571	Flavimonas-oryzihabitans	Flavimonas oryzihabitans or Chryseomonas luteola
407A20	0.66245669	Bacillus-pumilus-GC subgroup B	Bacillus genus
408S3	0.509538142	Bacillus-licheniformis	sim index too low
409S3	0.789240927	Bacillus-cereus-GC subgroup A	Bacillus cereus
410Ss3	0.743174592	Brevibacillus-reuszeri	Brevibacillus reuszeri
411Ss3	0.56809153	Bacillus-megaterium-GC subgroup A	sim index too low
412Ss3	0	NO MATCH	no match
413sS3			not tested
414Ss3	0.860571497	Citrobacter-freundii	Citrobacter freundii
415Ss3	0.715952104	Pectobacterium-carotovorum-carotovorum	Pectobacterium carotovorum carotovorum

416Ss3	0.64609415	Kocuria-kristinae-GC subgroup B	Enterobacteriaceae
417Ss3	0.494862884	Proteus-vulgaris	Enterobacteriaceae
418Ss3	0.570208881	Bacillus-megaterium-GC subgroup A	sim index too low
419Ss3	0.26174472	Pseudomonas-putida-biotype A	sim index too low
420Ss3	0	NO MATCH	no match
421Ss3	0.551095741	Bacillus-cereus-GC subgroup A	sim index too low
422Ss3	0.131255069	Grimontia-hollisae	sim index too low
423S4	0.680020454	Pectobacterium-carotovorum-carotovorum	Pectobacterium carotovorum carotovorum
424S4	0.975262477	Brevundimonas-vesicularis	Brevundimonas vesicularis
425S4	0.474660052	Bacillus-megaterium-GC subgroup A	sim index too low
426S4	0.716067501	Bacillus-thuringiensis-canadensis	Bacillus thuringiensis
427S4	0.205574392	Grimontia-hollisae	sim index too low
428S4	0.626796018	Bacillus-pumilus-GC subgroup B	Bacillus pumilus
429S7	0.881021463	Bacillus-cereus-GC subgroup A	Bacillus genus
430S7	0.789789887	Bacillus-atrophaeus	Bacillus atrophaeus or Bacillus subtilis
431S7	0.335098201	Acinetobacter-calcoaceticus	sim index too low
432S7	0.494709615	Bacillus-mycoides-GC subgroup B	sim index too low
433S7	0.546634747	Bacillus-megaterium-GC subgroup A	sim index too low
434S7	0.947328179	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
435S7	0	NO MATCH	no match
435-1S7			not tested
435-2S7r	0	NO MATCH	no match
436S7	0.385587546	Acinetobacter-calcoaceticus	sim index too low
437S7	0.794876587	Cedecea-davisae	Cedecea davisae
438S7	0.412825641	Ewingella-americana	sim index too low
439S7	0.844379978	Pantoea-ananatis/Erwinia uredovora	Pantoea ananatis/Erwinia uredovora
440S7	0.299195637	Leuconostoc-mesenteroides-dextranicum	sim index too low
441S7	0.610808509	Pantoea-agglomerans-GC subgroup B	Enterobacteriaceae
442S7	0	NO MATCH	no match
443S7	0.784690093	Bacillus-sphaericus-GC subgroup A	Bacillus sphaericus
444S7	0.28454928	Grimontia-hollisae	sim index too low

445S7	0.319017263	Grimontia-hollisae	sim index too low
446S10	0.548966038	Bacillus-mycoides-GC subgroup B	sim index too low
447S10	0.506317095	Pseudomonas-putida-biotype B/vancouverensis	sim index too low
448S10			not tested
449S10			not tested
450S10	0.320561512	Microbacterium-barkeri	sim index too low
450-1S10r	0.95425686	Bacillus-megaterium-GC subgroup A	Bacillus megaterium- not same as original
450-2S10	0.938248725	Bacillus-megaterium-GC subgroup A	Bacillus megaterium- not same as original
451S10	0.887893038	Sphingomonas-sanguinis	Sphingomonas sanguinis
452Ss12	0.820082418	Bacillus-thuringiensis-kurstakii	Bacillus genus
453Ss12	0.712005717	Pseudomonas-putida-biotype B/vancouverensis	Pseudomonas putida
454Ss12	0.529630642	Bacillus-pumilus-GC subgroup B	sim index too low
455Ss12	0.696668525	Escherichia-coli-GC subgroup B	Escherichia coli
456Ss12	0.885161702	Microbacterium-barkeri	Microbacterium barkeri
457Ss12	0.710036431	Escherichia-coli-GC subgroup B	Escherichia coli
458Ss12	0.351482817	Kurthia-gibsonii	Enterobacteriaceae
459Ss12	0.480280883	Bacillus-cereus-GC subgroup A	sim index too low
460Ss12	0.69761819	Pantoea-agglomerans-GC subgroup C	Enterobacteriaceae
461sS12	0.184984723	Stenotrophomonas-acidaminiphila	sim index too low
462Ss12	0.790942484	Salmonella-bongori/enterica	Enterobacteriaceae
463Ss12	0.860723973	Escherichia-coli-GC subgroup D	Enterobacteriaceae
464Ss12	0.608599675	Bacillus-pumilus-GC subgroup B	Bacillus genus
465Ss12	0.623985895	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
466-1Ss12	0.925647422	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
466-2Ss12	0.551985119	Microbacterium-barkeri	sim index too low
467Ss12	0.789560871	Escherichia-coli-GC subgroup B	Enterobacteriaceae
468S13	0.585635952	Serratia-plymuthica	Enterobacteriaceae
469S14	0.573310407	Bacillus-pumilus-GC subgroup B	sim index too low
470S14	0.644431084	Bacillus-pumilus-GC subgroup B	Bacillus pumilus
471S14	0.557196056	Bacillus-megaterium-GC subgroup A	sim index too low
472S14	0.651434405	Bacillus-cereus-GC subgroup A	Bacillus genus

473S14	0.886010827	Bacillus-cereus-GC subgroup A	Bacillus genus
474-1S14	0.867355992	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
474bS14	0.72552098	Bacillus-cereus-GC subgroup A	Bacillus cereus
475S13			not tested
476A13	0	NO MATCH	no match
476A13	0	NO MATCH	no match
476A13cr	0.1083368	Brevundimonas-diminuta	sim index too low
476A13c	0	NO MATCH	no match
477S13	0	NO MATCH	no match
478S13	0.688816993	Staphylococcus-saprophyticus	Staphylococcus genus
479S13	0.561150369	Serratia-plymuthica	Enterobacteriaceae
480S21	0.717471347	Salmonella-enterica-enterica E	Enterobacteriaceae
481S21	0.72215361	Pantoea-agglomerans-GC subgroup C	Enterobacteriaceae
482S21	0.776085935	Kluyvera-ascorbata-GC subgroup B	Kluyvera ascorbata
483S21	0.746343022	Kluyvera-cryocrescens-GC subgroup B	Enterobacteriaceae
484S21	0.756887815	Serratia-liquefaciens	Enterobacteriaceae
485S21	0.724464732	Serratia-liquefaciens	Enterobacteriaceae
486S21	0.704734621	Serratia-plymuthica	Enterobacteriaceae
487S21	0.70772336	Serratia-plymuthica	Enterobacteriaceae
488S21	0.734354836	Serratia-plymuthica	Enterobacteriaceae
489O3	0.761814444	Ewingella-americana	Ewingella americana
490O3	0	NO MATCH	no match
491O3	0.830988586	Sphingomonas-sanguinis	Sphingomonas sanguinis
492-1O3	0.420034684	Pseudomonas-savastanoi-nerium	sim index too low
492-2O3	0.819977607	Ewingella-americana	Ewingella americana
493O3	0.727537611	Ewingella-americana	Ewingella americana
494O3	0.579417022	Bacillus-pumilus-GC subgroup B	sim index too low
495-1O3	0.523076496	Sphingobium-yanoikuyae	sim index too low
495-2O3	0.277201911	Sphingobium-yanoikuyae	sim index too low
496-1O3	0.561836548	Sphingomonas-capsulata	sim index too low
496-2O3	0.497712122	Sphingomonas-capsulata	sim index too low
497O3	0.114051251	Brevundimonas-diminuta	sim index too low
498O3	0.755888961	Ewingella-americana	Ewingella americana
499O3	0.861185479	Sphingomonas-sanguinis	Sphingomonas

			sanguinis
500O7	0.721997189	Bacillus-thuringiensis-canadensis	Bacillus genus
501O7	0.789220829	Paenibacillus-polymyxa	Paenibacillus polymyxa
			not tested
503O9	0	NO MATCH	no match
504O9	0.49197874	Bacillus-mycoides-GC subgroup B	sim index too low
504-1O9	0.461894883	Bacillus-mycoides-GC subgroup B	sim index too low
504-2O9	0.409588161	Bacillus-cereus-GC subgroup A	sim index too low
505O9	0.540853612	Bacillus-pumilus-GC subgroup B	sim index too low
506O9	0.514641875	Bacillus-megaterium-GC subgroup A	sim index too low
507O9	0.496663771	Bacillus-megaterium-GC subgroup A	sim index too low
508O9	0.567163646	Arthrobacter-nicotianae-GC subgroup C	sim index too low
509O9	0.567807875	Bacillus-megaterium-GC subgroup A	sim index too low
510O9	0	NO MATCH	no match
511O9	0.491174312	Bacillus-cereus-GC subgroup A	sim index too low
512O11	0.572654355	Ewingella-americana	sim index too low
513O11	0.630598109	Microbacterium-barkeri	Microbacterium barkeri
514O11	0.692302482	Microbacterium-barkeri	Microbacterium barkeri
515O11	0	NO MATCH	no match
516O11	0.742194921	Bacillus-atrophaeus	Bacillus atrophaeus
517O11	0.7866859	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
518O12	0.836891156	Bacillus-subtilis	Bacillus subtilis or Bacillus atrophaeus
519O12	0.499978346	Bacillus-megaterium-GC subgroup A	sim index too low
520O12	0.868407721	Bacillus-megaterium-GC subgroup A	Bacillus megaterium
521Os12	0	NO MATCH	no match
522Os12	0.584886674	Bacillus-sphaericus-GC subgroup F	sim index too low
523Os12	0.147099754	Acinetobacter-calcoaceticus	sim index too low
524O12	0.13873719	Brevundimonas-diminuta	sim index too low
525O13	0.789816113	Acetobacter-pasteurianus	Acetobacter pasteurians
526O13	0.114998821	Brevundimonas-diminuta	sim index too low
527-1O13	0.269305012	Brevundimonas-diminuta	sim index too low
527-1O13r	0.194886289	Brevundimonas-diminuta	sim index too low

527-2O13	0.223092995	Lactobacillus-fermentum-GC subgroup A	sim index too low
527-3O13	0.611547293	Arthrobacter-nicotianae-GC subgroup C	Arthrobacter nicotianae
527-4O13	0.157232723	Corynebacterium-diphtheriae-intermedius	sim index too low
529O16	0.796530683	Bacillus-sphaericus-GC subgroup C	Bacillus sphaericus
530O16	0.916911639	Bacillus-cereus-GC subgroup A	Bacillus cereus
531O16	0.513990747	Bacillus-megaterium-GC subgroup A	sim index too low
532-1O16	0.803035547	Bacillus-cereus-GC subgroup A	Bacillus cereus
532-2O16	0.819072776	Bacillus-cereus-GC subgroup A	Bacillus cereus
533O16	0.845547349	Bacillus-cereus-GC subgroup A	Bacillus cereus
534O16	0.651297043	Ewingella-americana	Ewingella americana
535O18	0.793054229	Serratia-odorifera	Enterobacteriaceae
536-1O18	0.881925746	Microbacterium-lacticum-GC subgroup B	Enterobacteriaceae
536-2O18	0.623461517	Ewingella-americana	Ewingella americana
537O20	0.110833572	Bacillus-cereus-GC subgroup A	sim index too low
538O20	0.630256043	Bacillus-mycoides-GC subgroup B	Bacillus mycoides
539O20	0.828259571	Bacillus-cereus-GC subgroup A	Bacillus cereus
540O21	0.710683888	Rhodococcus-equi-GC subgroup B/Corynebacterium-hoagii	Rhodococcus equi
541O21	0.846919341	Bacillus-cereus-GC subgroup A	Bacillus cereus
542O21	0.216608153	Streptomyces-biverticillatus	sim index too low
543O21	0	NO MATCH	no match
544O16	0.536655835	Bacillus-megaterium-GC subgroup A	sim index too low
545O16	0	NO MATCH	no match
546O16	0.22596873	Bacillus-cereus-GC subgroup A	sim index too low
547O10	0	NO MATCH	no match
548O10	0.445564346	Acinetobacter-calcoaceticus	sim index too low
549O10	0.813346403	Pseudomonas-aeruginosa-GC subgroup A	Pseudomonas aeruginosa or Flavimonas oryzihabitans