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CURJ

The Cornell Undergraduate Research Journal

Expanding, Characterizing, and Repurposing *the* Invertebrate Virosphere

Variation in North American Rock Pigeons

Pigeons adapt to their local environment over time

Twitter Analysis of Pseudoarchaeology

A network analysis of archeology conspiracy theories and their consequences

Co-Diversification of Camponotus and Blochmannia

New evidence for co-diversification in bacteria and its ant hosts

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CORNELL UNDERGRADUATE RESEARCH JOURNAL

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Letter from the Editor

Dear Reader,

We are thrilled to present the second issue of the Cornell Undergraduate Research Journal (CURJ). CURJ is Cornell's peer-reviewed bi-annual research publication featuring the exemplary scholarly work of Cornell undergraduates across all disciplines and fields. The goal of this publication is to provide a platform for Cornell undergraduate students to showcase their research articles to the student body and to the general public, and offer the student body a wide range of academic perspectives, provoking intellectual pursuits and collaboration.

As we publish our second issue, this is a good time to reflect on the release of our first issue. I am happy to say that our first issue was a success. We accomplished our goal of publishing research papers of Cornell undergraduates across a wide range of disciplines with each article undergoing an extensive review process. We received lots of positive feedback and heard from many individuals who read the issue, commented on the articles, and discussed them with their peers. As an anecdote, once several members of the editorial team and I met a student at a fair. When he found out that we were working on CURJ, he told us that he read our first issue and disagrees with the premise of one of the articles, and explained his views on the matter. I was thrilled to hear this. This is exactly why we established this journal. Our goal is not only to share research articles, but also to provoke intellectual discussions and debates, to start dialogue among Cornell's research community, and to inspire more research that will ultimately advance knowledge even further.

I would like to thank our amazing, passionate, and hard-working team. This past year our team has been working relentlessly at building this publication from the ground up and has risen to the many challenges along the way. We welcomed new members to our team this Fall who have already greatly contributed to this issue. We are lucky to have such a skillful and dedicated team.

Thank you as well to our authors for their fantastic work on their research articles and to our graduate student reviewers and faculty advisors for their insightful feedback and contributions that have greatly enriched this publication. We would also like to thank Mike Priehs of the Cornell University Library for hosting and supporting our jurnal. And of course we would like to express our gratitude to Ellen Hartman, Research Communications Director of Cornell Research and our organization's advisor, without whom this publications would not have been possible.

We are very excited to share this second issue of the Cornell Undergraduate Research Journal and to present the exceptional research of Cornell undergraduates.

Sincerely,

Victoria Alkin

Victoria Alkin
Founder and Editor-In-Chief

CURJ

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Expanding, Characterizing, and Repurposing the Invertebrate Virosphere

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Abstract

Modern medicine demands the capacity to deliver genetic or biological cargo to specific cell types. Past efforts to achieve this goal have relied on the retooling and re-engineering of a small subset of vertebrate viruses with limited success. Remaining challenges with regards to in vivo delivery include finding novel viral vectors that can achieve different target specificities in addition to those that are more amenable to synthesize de novo. In an attempt to address these remaining limitations, we collected and sampled diverse invertebrate species to isolate and identify RNA viruses associated with them. As the invertebrate virosphere remains largely unknown, we hypothesized that we would identify novel viruses whose components could be characterized and repurposed to build a new suite of viral-based tools. To this end, we isolated and sequenced RNA from a diverse library of invertebrates (including 42 insects) by next-generation sequencing and subsequently performed de novo genome assembly on the reads obtained. Captured reads were analyzed for signatures of RNA dependent RNA polymerases (RdRps) – a necessary component of all RNA viruses. The two putative novel virus genome assemblies discovered were named Castor and Pollux, and were characterized and independently confirmed by quantitative PCR. These small RNA viruses or their RdRps (less than 5kB) will, in the future, be synthesized and artificially launched in mammalian cells to ascertain whether they can be selected via guided evolution to function and deliver a desired genetic or biological cargo.

Introduction

In the advent of recombinant DNA technology, science has witnessed the development of tools to generate antibodies from a plasmid, silence messenger RNA, deliver genes, and even edit DNA with single base pair resolution (Khan et al., 2016). However, to capitalize on these discoveries, the scientific community needs the ability to deliver the necessary cargo to the cells of interest (Dobson, 2006). Thus far, the issue of delivering genetic material to cells has come in the form of either repurposed viral vectors or the direct delivery of genetic material (Thomas et al., 2003).

With regards to viral vectors, these have largely focused on the use of lentiviruses, adenoviruses, or the adeno-associated virus (Robbins & Ghivizzani, 1998). While each of these viral vectors has demonstrated promise in some context, each has inherent issues preventing their widespread use. For example, early clinical trials with lentiviruses resulted in multiple integration events that culminated in the development of cancer (Condiotti et al., 2014). Conversely, use of an adenovirus, while it does not integrate, many in the human population have pre-established immunity to the vector rendering it ineffective. While both seroprevalence and integration are issues that can be addressed with additional testing or methodology, neither vector represents a tool that can be used in a more generalized platform (Vemula & Mittal, 2010). In place of these two popular vectors, a third expression system has gained popularity called the Adeno-associated virus (AAV). This vector, which in nature impacts a wide variety of animal species, can be repurposed for gene delivery and has shown significant promise in clinical trials (Naso et al., 2017). While AAV neither integrates nor shows high seroprevalence, its limitations derive from the fact that it tends to deliver to the liver and has a very limited coding capacity (Robbins & Ghivizzani, 1998).

While we still use these vectors, the limitations are well understood and the scientific community is simultaneously looking for other solutions. Over the past few years, many researchers have focused on various lipids or synthetic nanoparticles to deliver recombinant DNA to cells (Zhao & Huang, 2014). However, this has proven difficult, largely owing to the inability to breach the barriers required to reach the nucleus. More recently, a promising technology in this area is the direct use of RNA. The use of RNA as a therapeutic is promising in that it can be easily manufactured and does not integrate. However, while promising, a remaining limitation of RNA is its inherent instability. In this regard, the identification of novel RdRps may also enable the engineering of self-replicating RNAs, thereby overcoming this limitation (Lundstrom, 2021). In an effort to find a small RdRp that will not show any prevalence in the human population, we sought to sample invertebrates for novel RNA viruses from which we could build, in two significant steps:

1. The initial aim of this project was to gather RNA samples from variegated sources. Multiple samples from a wide range of invertebrate species provided the necessary heterogeneity from which RNA was isolated. Subsequent construction of a diverse invertebrate RNA library allowed for the identification and classification of viruses present within each sample (regardless of genome type). The RNA library was then used to sequence, assemble and identify putative viruses.

2. Identification of a novel virus was immedately followed by a thorough characterization and analysis of open reading frames (ORFs). Compatibility with cloning and evolutionary relationships to other known viruses can then be assessed. As previously stated, small RNA viruses and/or viral RdRps that neither integrate nor have a high seroprevalence are ideally suited to work with and advance. Subsequent cloning via synthetic biology and launching in permissive cell lines serve as the next steps in the progression and development towards a self-replicating RNA.

Methods and Materials

RNA Isolation from Collected Samples

To address the first aim, insects were collected and stored in RNALater® from predetermined environments in a set area. We recorded the sample ID and suspected species using www.amentsoc.org/insects/what-bug-is-this/. The collected insect was then pulverized with small quantities of TRIzol reagent, with the exact amounts dependent on total sample size. Subsequent incubation allowed for the phenol in TRIzol to break down cellular components while maintaining RNA integrity. Chloroform was added to the solubilized RNA to induce phase separation, which occurred over a fifteen minute period of centrifugation. The generated supernatant contained RNA in the colorless upper aqueous phase and was transferred out of solution via pipetting. The red organic proteinaceous layer and DNA interphase layer were discarded. A quantity of isopropanol, equal to half the added amount of TRIzol Reagent, was mixed into the aqueous solution and allowed to incubate, and the insolubility of RNA in isopropanol yielded a white RNA pellet, albeit impure. Subsequent resuspension in 80% ethanol allowed for purification of the RNA due to ethanol's low dielectric constant and propensity of the salt to dissolve in water and force it out from the RNA. The remaining pellet of RNA was then characterized using a Nanodrop instrument®. This RNA was cataloged and stored at -80°C.

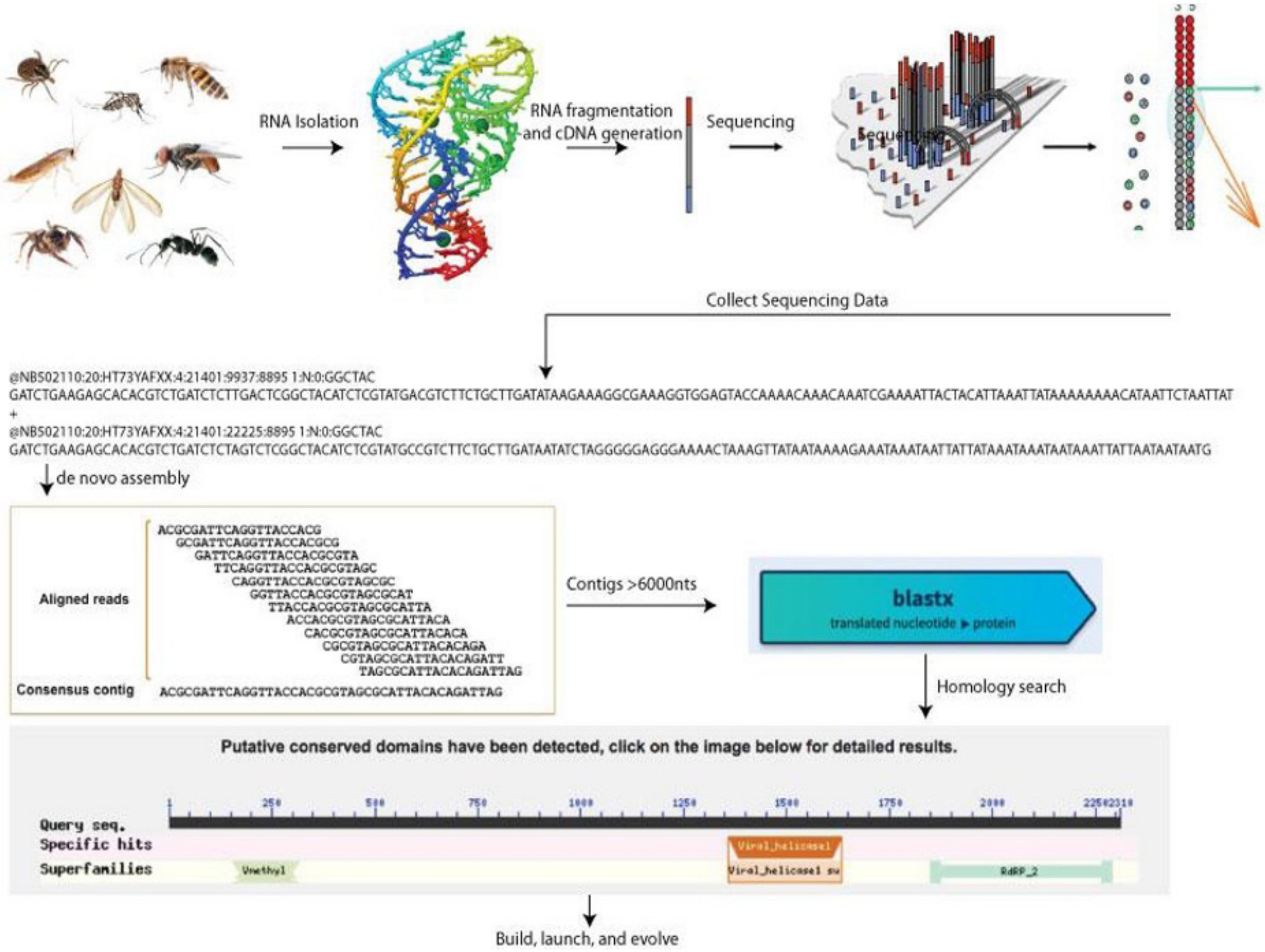
Next Generation Sequencing

High-quality RNA isolated from the previous step (as determined by the Nanodrop) was fragmented and used to generate an Illumina-compatible library for massively-parallel sequencing (see Figure 1). The process followed in this procedure was informed by Michael Quail's literature on the topic (Quail et al., 2009). In brief, each captured

RNA fragment was used to amplify an isolated pool of identical cDNA fragments that could be sequenced alongside each other. Using a high-resolution camera and real-time primer-mediated extension, the NextSeq Illumina instrument can generate 500 million reads from a single run. RNA samples were, therefore, cloned and processed in this way and sequenced and assembled de novo to identify contiguous RNAs that were greater than 6000 nts in length (as this exceeds the size of most mRNAs whereas viruses are commonly larger than this). "Contigs" were then translated in all 6 possible frames and putative proteins (larger than 600aa) were used in a BLASTx search to determine whether there was any homology to known RNA dependent RNA polymerases (RdRps), which are generally larger than 600 residues. Sequenced contigs showing homology to known RdRp were then characterized to identify additional open reading frames (ORFs). Each putative ORF was aligned to known viruses and fitted into a phylogenetic tree to ascertain which family of viruses it was contained within.

Viral Selection

Based on our final list of putative viruses, we prioritized which ones we move forward with using a number of criteria. First, it was essential to have high genetic coverage of the genome to be certain of the viral sequence. For this reason,



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@NB502110:20:HT73YAFXX:4:21401:9937:8895 1:N:0:GGCTAC
GATCTGAAGAGCACACGTCTGATCTCTTGACTCGGCTACATCTCGTATGACGTCTTCTGCTTGATATAAGAAAGGCGAAAGGTGGAGTACCAAAACAAACAAATCGAAAATTACTACATTAAATTATAAAAAAAACATAATTCTAATTAT
+
@NB502110:20:HT73YAFXX:4:21401:22225:8895 1:N:0:GGCTAC
GATCTGAAGAGCACACGTCTGATCTCTAGTCTCGGCTACATCTCGTATGCCGTCTTCTGCTTGATAATATCTAGGGGGAGGGAAAACTAAAGTTATAATAAAAGAAATAAATAATTATTATAAATAAATAATAAATTATTAATAATAATG
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ACGCGATTCAGGTTACCACG
  GCGATTCAGGTTACCACGCG
    GATTCAGGTTACCACGCGTA
      TTCAGGTTACCACGCGTAGC
        CAGGTTACCACGCGTAGCGC
          GGTTACCACGCGTAGCGCAT
            TTACCACGCGTAGCGCATTA
              ACCACGCGTAGCGCATTACA
                CACGCGTAGCGCATTACACA
                  CGCGTAGCGCATTACACAGA
                    CGTAGCGCATTACACAGATT
                      TAGCGCATTACACAGATTAG
ACGCGATTCAGGTTACCACGCGTAGCGCATTACACAGATTAG
```

Figure 1: Animation Depicting the Overall Strategy of Data-Collection. The process begins with the collection of invertebrates. The samples then undergo RNA isolation in order for us to sequence them. In the sequencing step, the RNA is fragmented into many pieces before being amplified many times. The fragments will contain overlapping segments of genetic code, making them amenable to reconstruction via de novo assembly. The subsequent consensus contigs vary in length, but the longer ones (>6000 nucleotides (nts)) will be analyzed via Basic Local Arrangement Search Tool (BLAST) to contrast them with known viruses and determine if they are novel viruses. Putative small viruses and/or RdRps will then be synthesized and introduced into mammalian cells to determine if we can select them to function (this step is denoted as Build, launch, and evolve).

we only built viruses that have greater than 10x coverage across the genome at every position. Second, we prioritized viruses that are novel. And third, we chose the smallest viruses that fulfilled the above criteria as they can be synthesized relatively easily. Should we focus on a virus of positive polarity (which can be determined by RdRp homology) we would transcribe RNA and introduce it into cells for further study. Should we discover a virus of negative polarity, we would clone the polymerase into a plasmid to enable host production prior to introducing the genomic RNA for further study.

Results

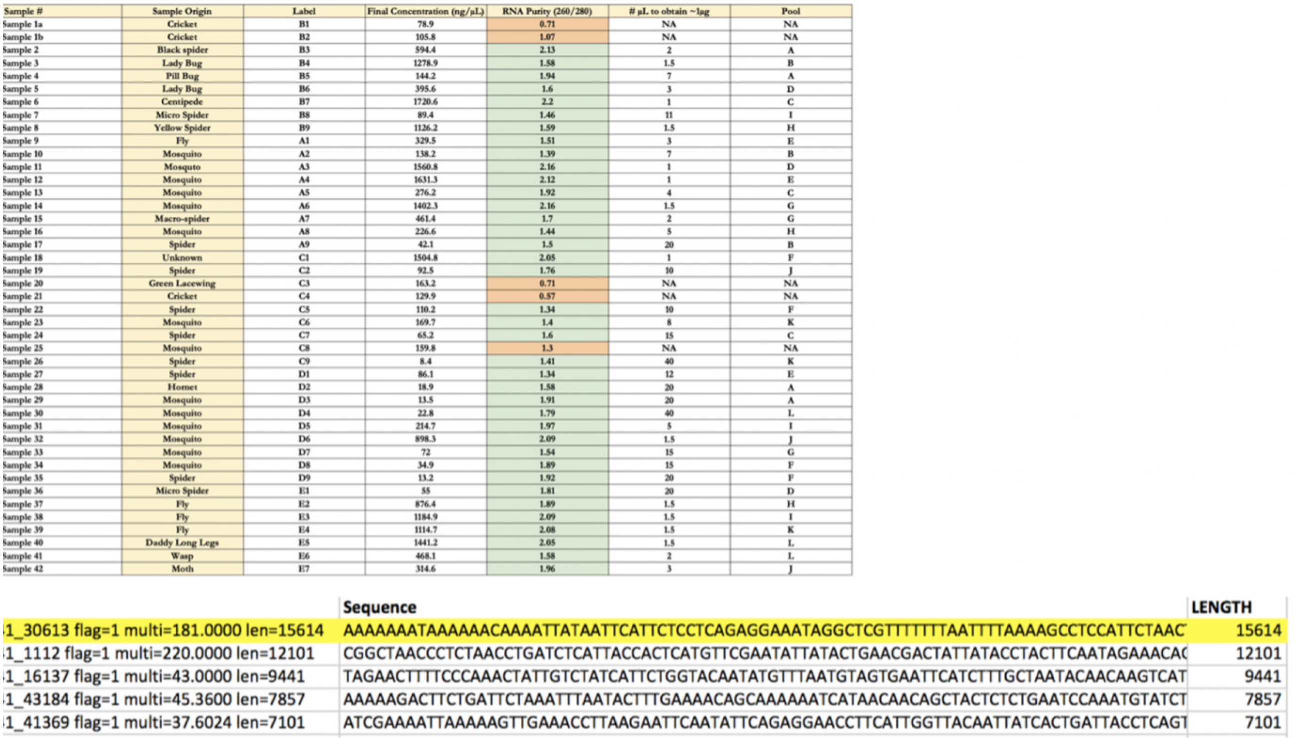
The first step in interpreting the data involved construction of an RNA library generated from our diverse collection of arthropod and arachnid species. The RNA of 42 individual insects were sampled and analyzed throughout the duration of the project. One insect, the cricket, was split into two sections, for a total of 43 samples. Figure 2a tabulates information about each of these samples, including the insect of origin and the label associated with it. The RNA concentrations and purity are also shown. Of the 43 samples, five were rendered impotent by RNA purities that were too low to sequence. These samples are highlighted red in figure 2a. These results could have been due to human error in the isolation process, or the lack of a quantifiable amount of RNA in the insect genome. The 38 remaining samples were split into twelve different pools for sequencing, labeled A through L. The methodology behind splitting the samples consisted of organizing groups of samples with no overlap between insect types and pairing samples with lower RNA yields to those with higher yields. Loose approximations were made to mix ~1μg of each sample into each pool, for a total of ~3μg in a 100μl solution. Next Generation Sequencing was then performed following the outline described previously. The subsequent sequencing results were organized in an excel spreadsheet by length, and nine out of twelve pools contained “contigs” of greater than 6000 nucleotides. Contigs of this length or greater were considered potential viral candidates, while shorter contigs were disregarded. These remaining contigs were compared against existing libraries of RNA samples to determine if they were viral, and if so, whether they were novel. BLASTx revealed that the vast majority of the contigs had high homology to known viral or other RNA-containing species. In fact, in eleven out of twelve pools, none of the contigs were novel viruses. In Pool D however, two novel viruses were identified and were named Castor and Pollux. Figure 2b reveals a sample output of BLASTx for the longest contigs in Pool D. The longest of these contigs, with a length of 15614 base pairs, was Castor. The second longest, with a length of 12101 base pairs, was Pollux.

Characterization of Castor and Pollux:

Castor and Pollux were identified as viral RNAs by BLASTx because they contained segments with homology to known RNA dependent RNA polymerases (RdRps). These RdRps are essential proteins encoded by RNA viruses that have no DNA stage, and are thus a good but fallible indicator of viral identity. The viral RNAs were thus further characterized to identify additional ORFs. Figure 3a shows the five ORFs identified for Castor. The RdRp segment codes for the RNA-dependent RNA polymerase, and is the longest ORF at 7089bp. It codes for a protein with a Mw upwards of 270da. The nucleoprotein (NP) was identified due to homology with other viral NPs. With a length of 1479bp, it codes for a protein that encapsidates the viral genome and is a necessary element of all negative-sense RNA genomes. Also notable is the spike protein, which is almost certainly involved in penetration and infection of host cells. Figure 3b similarly shows the ORFs of Pollux. Like Castor, there are five identifiable ORFs, and with the exception of the ORF2 (the ORF coding for the spike protein in Castor), the ORFs in Castor and Pollux seem to be well aligned. Figure 3c, which shows the molecular weights of the ORFs, hints at a potential relationship between the viruses since the molecular weights of the RdRp and NP sections are similar. In order to confirm the existence of each of these ORFs, primers were designed and they were amplified and run through a gel. Figure 3d shows the result of one gel run for the Castor

ORFs as an example output; however, every ORF was individually separated and confirmed successfully.

Both RNAs were determined to be single-stranded, negative-sense viruses, and both originated from the family of Rhabdoviridae. Each putative ORF was aligned to known viruses to establish these viral relationships within a phylogenetic tree. Sample outputs of these trees, based on the RdRps of the two viruses, are shown in Figure 4. The nearest relation for both RdRps is an unclassified Coleopteran rhabdovirus. The similar results for each pair of ORFs, in addition to a 43% global homology rating between the two viruses, suggest a relationship and likely a common ancestor. Although confirmation of their origins was not determined, homology searches suggest that these viruses came from the same insect. Based on the loosely conserved RNA sequences present in the sample, we hypothesize that these viruses came from the only spider in pool D (sample #36). It is possible, however, that they came from different insects within the same pool. It is due to their similarity to each other that the two viruses were named after the twins from Greek mythology, Castor and Pollux.



Sample #	Sample Origin	Label	Final Concentration (ng/µL)	RNA Purity (260/280)	# µL to obtain ~1µg	Pool
Sample 1a	Cricket	B1	78.9	0.71	NA	NA
Sample 1b	Cricket	B2	105.8	1.07	NA	NA
Sample 2	Black spider	B3	594.4	2.13	2	A
Sample 3	Lady Bug	B4	1278.9	1.58	1.5	B
Sample 4	Pill Bug	B5	144.2	1.94	7	A
Sample 5	Lady Bug	B6	395.6	1.6	3	D
Sample 6	Centipede	B7	1720.6	2.2	1	C
Sample 7	Micro Spider	B8	89.4	1.46	11	I
Sample 8	Yellow Spider	B9	1126.2	1.59	1.5	H
Sample 9	Fly	A1	329.5	1.51	3	E
Sample 10	Mosquito	A2	138.2	1.39	7	B
Sample 11	Mosquto	A3	1560.8	2.16	1	D
Sample 12	Mosquito	A4	1631.3	2.12	1	E
Sample 13	Mosquito	A5	276.2	1.92	4	C
Sample 14	Mosquito	A6	1402.3	2.16	1.5	G
Sample 15	Macro-spider	A7	461.4	1.7	2	G
Sample 16	Mosquito	A8	226.6	1.44	5	H
Sample 17	Spider	A9	42.1	1.5	20	B
Sample 18	Unknown	C1	1504.8	2.05	1	F
Sample 19	Spider	C2	92.5	1.76	10	J
Sample 20	Green Lacewing	C3	163.2	0.71	NA	NA
Sample 21	Cricket	C4	129.9	0.57	NA	NA
Sample 22	Spider	C5	110.2	1.34	10	F
Sample 23	Mosquito	C6	169.7	1.4	8	K
Sample 24	Spider	C7	65.2	1.6	15	C
Sample 25	Mosquito	C8	159.8	1.3	NA	NA
Sample 26	Spider	C9	8.4	1.41	40	K
Sample 27	Spider	D1	86.1	1.34	12	E
Sample 28	Hornet	D2	18.9	1.58	20	A
Sample 29	Mosquito	D3	13.5	1.91	20	A
Sample 30	Mosquito	D4	22.8	1.79	40	L
Sample 31	Mosquito	D5	214.7	1.97	5	I
Sample 32	Mosquito	D6	898.3	2.09	1.5	J
Sample 33	Mosquito	D7	72	1.54	15	G
Sample 34	Mosquito	D8	34.9	1.89	15	F
Sample 35	Spider	D9	13.2	1.92	20	F
Sample 36	Micro Spider	E1	55	1.81	20	D
Sample 37	Fly	E2	876.4	1.89	1.5	H
Sample 38	Fly	E3	1184.9	2.09	1.5	I
Sample 39	Fly	E4	1114.7	2.08	1.5	K
Sample 40	Daddy Long Legs	E5	1441.2	2.05	1.5	L
Sample 41	Wasp	E6	468.1	1.58	2	L
Sample 42	Moth	E7	314.6	1.96	3	J

	Sequence	LENGTH
1_30613 flag=1 multi=181.0000 len=15614	AAAAAAATAAAAAACAAAATTATAATTCATTCTCCTCAGAGGAAATAGGCTCGTTTTTTTAATTTTAAAAGCCTCCATTCTAAC	15614
1_1112 flag=1 multi=220.0000 len=12101	CGGCTAACCCTCTAACCTGATCTCATTACCACTCATGTTCGAATATTATACTGAACGACTATTATACCTACTTCAATAGAAACA	12101
1_16137 flag=1 multi=43.0000 len=9441	TAGAACTTTTCCCAAACTATTGTCTATCATTCTGGTACAATATGTTTAATGTAGTGAATTCATCTTTGCTAATACAACAAGTCAT	9441
1_43184 flag=1 multi=45.3600 len=7857	AAAAAGACTTCTGATTCTAAATTTAATACTTTGAAAACAGCAAAAAATCATAACAACAGCTACTCTCTGAATCCAAATGTATCT	7857
1_41369 flag=1 multi=37.6024 len=7101	ATCGAAAATTAAAAAGTTGAAACCTTAAGAATTCAATATTCAGAGGAACCTTCATTGGTTACAATTATCACTGATTACCTCAGT	7101

Figure 2: An Overview of the Samples and Initial Data. a, Tabular data showing which insects the 43 samples originated from and the pools into which they were grouped. Note that one insect, the cricket, was large and therefore split into samples 1a and 1b. **b,** Sample data showing some results for Pool D. Each pool generated similar data, with hundreds of contigs of varying lengths. The highlighted segment denotes the automatically generated ID, nucleotide sequence, and length for the first contig, which was the novel virus dubbed "Castor."

Discussion

Viruses are omnipresent, and yet many viral genomes are unrecognized and undocumented. This project has demonstrated, first and foremost, the potential of next-generation sequencing and de novo genome assembly to expand the invertebrate virosphere. However, despite the discovery and analysis performed in this study, much work remains to be done. After all, with the discovery of two novel viruses comes the introduction of a new suite of tools that could be repurposed for gene editing. To develop such tools, we will first need to verify expression of Castor and Pollux within their cognate RNA samples to ensure and verify their sequences. The negative stranded character of the two viruses suggests the promise of cloning the polymerases into a

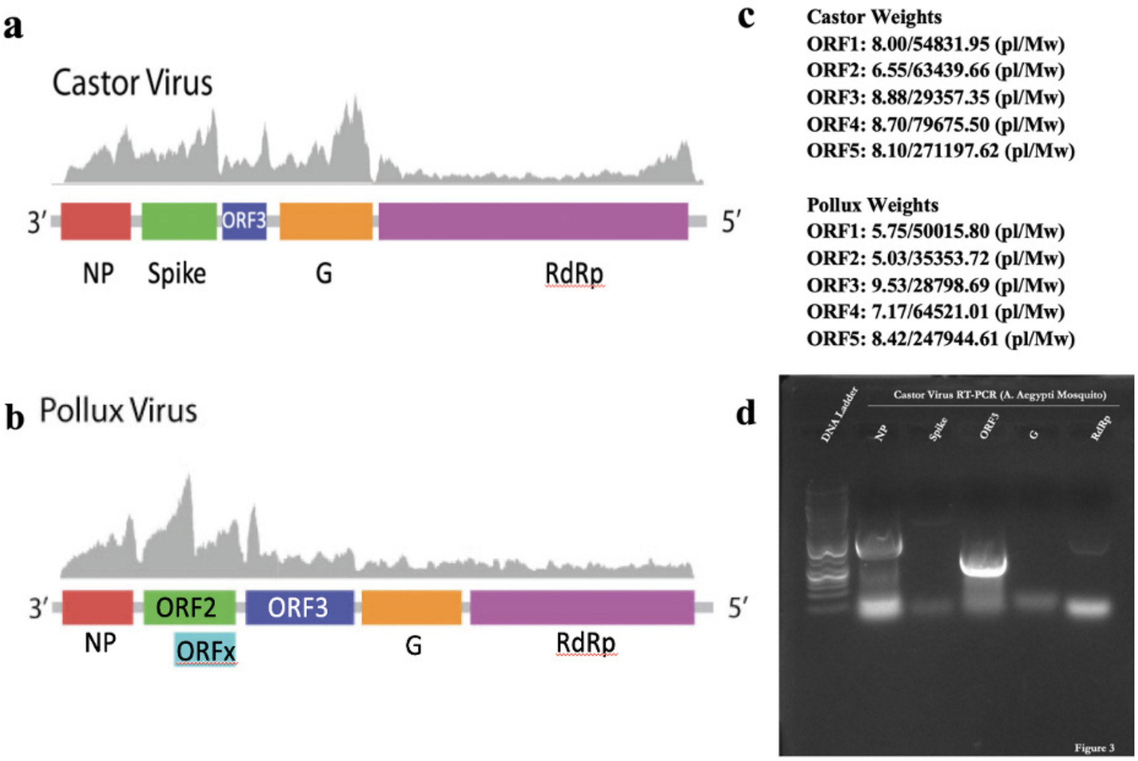


Figure 3: Open-reading Frames of Castor and Pollux. a, A breakdown of the ORFs identified in Castor. The RdRp is the RNA-dependent RNA polymerase, and NP is the nucleoprotein. Five ORFs were identified overall. **b,** A breakdown of the ORFs identified in Pollux. The RdRp and NP regions are similar to those in Castor. Five ORFs were identified overall, but there is no conventional spike protein like that found in Castor. **c,** Provides some information for each of the ORFs found in Castor and Pollux, notably the molecular weights of the corresponding proteins. **d,** An example gel demonstrating confirmation that these ORFs exist, are separable, and are well-defined.

plasmid, so PCR amplification and subcloning into plasmids suitable for in vitro transcription and/or eukaryotic expression is a direct next step. Enabling such host production will be followed by introducing the plasmid into insect or mammalian cells (C6/36 or BHK cells, respectively), and PCR can then be used to determine whether evidence of self-amplification can be observed. Should we see some levels of "replication," we will continue to passage the viruses to determine whether we can guide their activity and study their biology. Successful replication will, in the long term, be followed by additional analysis of the putative ORFs and isolation of the RdRps to qualitatively determine the potential of guided evolution to achieve a functional enzyme in mammalian cells.

Also notable about Castor and Pollux is their close relationship to each other. Initial analysis suggested only one viral discovery, but a closer look quickly demonstrated that two viruses with a high homology were in fact present. An interesting further study could test for interdependence between these two viruses. While it is well-known that viruses are fully dependent on host cell machinery in order to replicate, it would be a novel phenomenon for two viruses to also be dependent on each other.

Continuing to expand the virosphere should be a major scientific goal, and more effort should be put into identifying and characterizing new viral genomes. The fact that Castor and Pollux were discovered in such a small sample size suggests the large number of viruses yet to be discovered. Previous similar experiments have yielded many more viruses in even smaller populations. The invertebrate virosphere contains remarkable variety and flexibility as a result of the frequent rate of recombination and horizontal gene transfer. Continuing to take advantage of such rapid evolution and diversity has the potential to yield numerous novel therapeutic vectors. At the very least, continuing to find such viruses will continue to expand our knowledge of the virosphere and the diversity and mysteries it contains.

Conclusion

RNA viruses represent one of the greatest sources of biodiversity in the world, and yet knowledge of the many species and families remains limited. Our historical emphasis on studying viruses in cultures or as disease-causing agents has caused us to neglect large and diverse groups of more unremarkable populations. This study sought to begin to analyze one such population—the invertebrate virosphere. By isolating and sequencing the RNA from 42 insects, and creating a diverse RNA library via next-generation sequencing and de novo genome assembly, we were able to identify two novel viruses. These putative novel viral genomes were named Castor and Pollux, and were subsequently characterized and independently confirmed by quantitative PCR. Aligning the ORFs of the newly discovered viruses to preexisting counterparts allowed for the determination that they are single-stranded, negative-sense viruses from the family Rhabdoviridae. While much work remains to be done to achieve real medical progress, Castor and Pollux exemplify the unrecognized and underappreciated diversity and potential of RNA viruses, whose rapid evolution and variable genomic size, structure and segmentation make them wildly promising prospective candidates for various therapeutic applications. The data recovered from these pursuits will not only allow for the development of viral vectors and novel therapies, but will also inform our knowledge of the world around us and provide perspective on the evolutionary intricacies, patterns, and developments within the viral world.

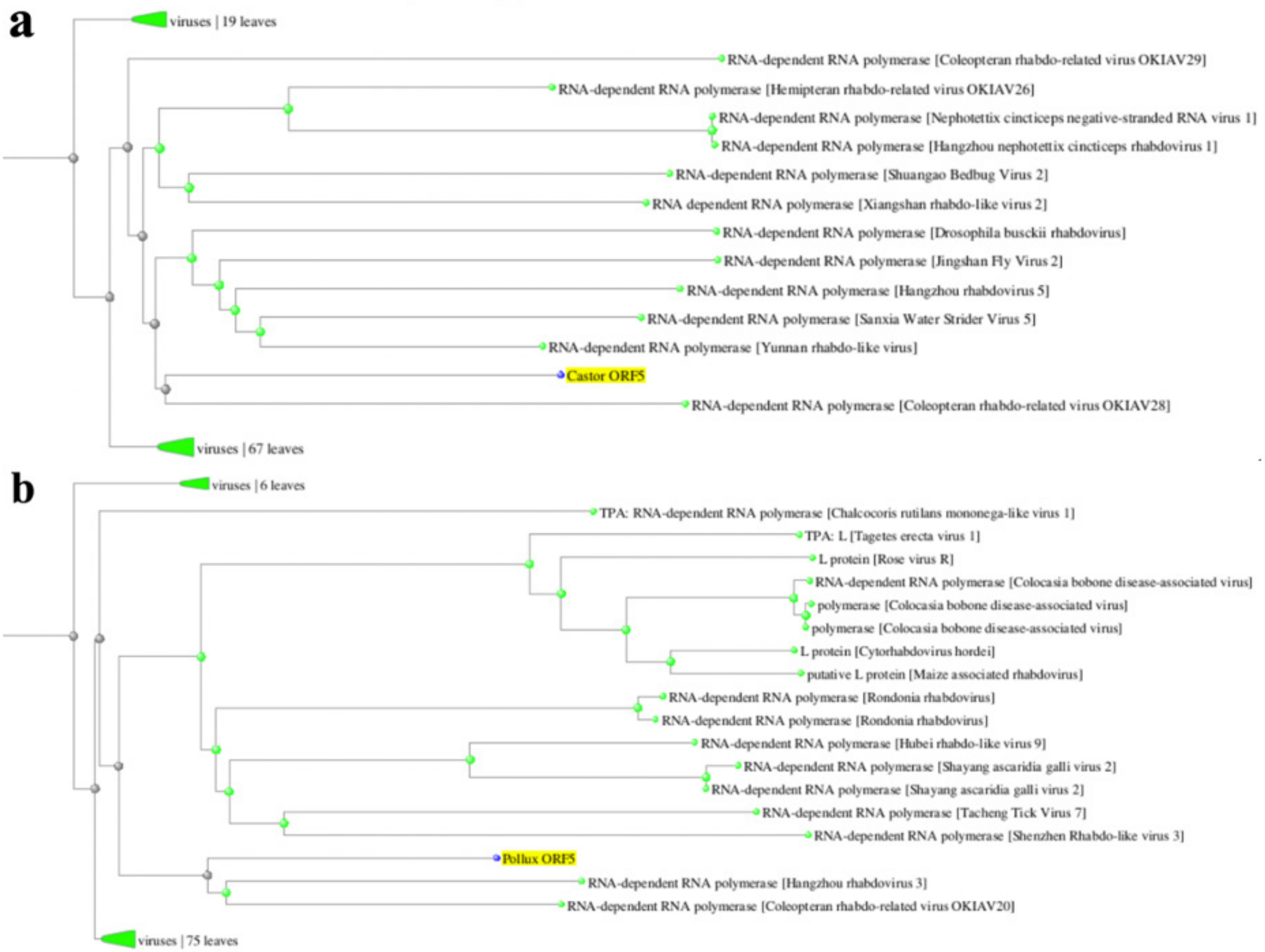


Figure 4: Sample Phylogenetic Trees for Novel Viruses. a, A neighbor-joining phylogenetic tree for Castor's RNA-dependent RNA polymerase (labelled Castor ORF5). **b,** A neighbor joining phylogenetic tree for Pollux's RNA-dependent RNA polymerase (labelled Pollux ORF5).

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Co-diversification of Camponotus and Its Primary Endosymbiont *Blochmannia* in the USA

By Steven Wang, Dr. Manuela de O. Ramalho
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Abstract

Blochmannia is a primary intracellular endosymbiotic bacteria found within the ant tribe Camponotini (Family *Formicidae*: subfamily *Formicinae*). *Blochmannia* is transmitted vertically through maternal host lineages and synthesizes essential amino acids for its host species. The high fidelity of vertical transmission observed means that close co-diversification is expected between *Blochannia* and their Camponotini hosts. This study expands on past data on USA Camponotini-*Blochmannia* associations and adds 23 new species to the host COI mitochondrial phylogeny and the 16S ribosomal DNA bacterial phylogeny. Using statistical analyses we found evidence of *Blochmannia* co-diversification with the host with enhanced species coverage, and one clear host shift is documented.

Keywords: horizontal transmission, phylogeny, symbiosis

Introduction

Many insects have 'primary' endosymbionts that are closely linked to host survival and fecundity. Primary endosymbionts often show very close diversification with their hosts, in which case their respective phylogenies will be congruent (Baumann & Baumann 2005, Moran et al. 1993, Munson et al. 1991, Spaulding & von Dohlen 2001, Thao et al. 2000). Vertical transmission can lead to co-diversification as host and endosymbiont. Taxonomic groups that have extensive co-diversification with primary symbionts are often much more diverse than their sister taxa, as in the case of the Order *Hemiptera* within the Superorder Acercaria (Johnson et al. 2018). Here we focus on the co-diversification of the endosymbiotic bacteria *Blochmannia* found in the ant tribe Camponotini (Family Formicidae: subfamily Formicinae).

There have been extensive studies on the co-diversification of bacteria and their ant hosts in another bacterial genus *Wolbachia*. Unlike *Blochmannia*, *Wolbachia* is found widely across Insecta and does not always have perfect vertical transmission, and does not show close co-diversification. Wolbachia is a commonly found endosymbiont in ants, including Camponotini (Russel 2012). There are many documented cases of horizontal *Wolbachia* transmission within Hymenoptera (Gerth et al. 2013, Raychoudhury et al. 2009, Tseng et al. 2019), including ants (Boivin et al. 2014, Frost et al. 2010, Tolley et al. 2019). This contrasts with *Blochmannia*, in which the existence of horizontal transmission has been suggested (Degnan et al. 2004) but has not been observed experimentally (Ramalho et al. 2017, Saur et al. 2000).

Blochmannia

Blochmannia is an endosymbiotic bacteria that resides in bacteriocytes (Saur et al. 2002) of its host ants. It was discovered to reside in the midgut lining of all castes and the ovarioles of *Camponotus* (Blochmann 1887, Saur et al. 2000). Their proximity to the ovarioles plays a direct role in their vertical transmission (Ramalho 2018). The common ancestor of *Blochmannia* was likely horizontally transferred from hemipterans (Gil et al. 2003, Rafiqi 2020, Ward et al. 2016, Wernegreen et al. 2009). They are endemic endosymbionts to the Camponotini clade of ants and likely play a nutritional synthesis role, providing hosts with vitamins (Degnan et al. 2005, Gil et al. 2003).

Nutritional endosymbionts are often found in herbivorous taxa and can help the host synthesize nutrients missing in their diet (Baumann et al. 2006, Feldhaar & Gross 2009). This can help the host species expand to new niches previously inaccessible due to a lack of nutrients. *Blochmannia* crosses over to the next generation in the egg (Rafiqi 2020, Ramalho et al. 2018), where they contribute to larval nutrition and survival, especially in the absence of essential amino acids (Feldhaar 2007). *Camponotus* are known to be omnivorous but have a lower $^{15}N/^{14}N$ ratio than other ants, which indicates a diet skewed towards herbivory (Moreau 2020). There is evidence that many *Blochmannia* supply their Camponotini hosts with riboflavin and vitamin B6 (Williams & Wernegreen 2015), which the ants may lack in their plant-heavy diet.

Perfect co-diversification between *Camponotus* and their endosymbiont *Blochmannia* has been observed in analyses with fewer species (Degnan et al. 2004). The purpose of this study is to generate new host and endosymbiont sequence data for USA Camponotini-*Blochmannia* relationships and use the larger dataset to test if co-diversification is still supported. To accomplish this, Camponotini were collected from the continental USA. We then sequenced and reconstructed phylogenies of host COI mitochondrial DNA and endosymbiont *Blochmannia* 16S rDNA using maximum-likelihood and Bayesian methods. COI mitochondrial and 16S rDNA genes were chosen for their known phylogenetic signal and cost-effectiveness (Mardulyn & Whitfield 1999, Nunez & Oleksiak 2016).

Materials and Methods

Collection of Specimens

Ants were collected during June 2021 from various sites in the USA. Ten species were collected by S. Wang from sites in the states of New Mexico, Texas, Louisiana, Mississippi, and Pennsylvania. An additional 11 species were collected by collaborators in Texas, Florida, and Arizona (see Table 1). Ants were collected directly into 95% EtOH. These samples were combined with the 16 species sequenced in Degnan et al. 2004, and unpublished data from Schluns et al., which brings this study to a total of 39 sequenced Camponotini. This makes up 62% of the 63 species of valid extant Camponotini species present in the continental USA. Vouchers were point mounted and identified to species with keys (Macgown n.d., Hansen & Klotz 2005, Snelling 2006, Snelling 1988) and deposited in the Cornell University Insect Collection (CUIC) under voucher numbers CUIC000007039-CUIC000007062.

DNA Isolation

Ant petiole + gaster segments were dissected for genetic analysis. These dissected samples were rinsed for 10 seconds each in 95% ethanol, 10% bleach, and pure water. Each cleaned sample was placed in a 1.5mL tube with a ceramic bead. The OMNI Bead Mill Homogenizer was used to homogenize the samples. Samples were checked visually, and sterile pipette tips were used to crush any remaining large tissue pieces. Vials were incubated with the lysis buffer at 56°C overnight in a VWR hybridization oven. Genomic DNA was then extracted from the samples using a Qiagen DNEasy™ DNA extraction kit (Qiagen Inc., Valencia, CA), according to the manufacturer protocols.

Polymerase Chain Reaction (PCR) Amplification

PCR reactions were performed in 0.2mL strip tubes, each with 5.5µL water, 12.5µL Taq PLUS Master Mix (Invitrogen, Waltham, MA), 2.5µL 10mM forward primer, 2.5µL 10mM reverse primer, 1.0µL bovine serum albumin (BSA) from Thermo Scientific (Thermo Scientific, Vilnius, LT), and 1.0µL DNA product from the Qiagen DNEasy™ (Qiagen Inc., Valencia, CA) extraction. CAS 7732-18-5 Molecular Biology Water was obtained from VWR Life Sciences (VWR Chemicals, LLC, Solon, OH) and UV sterilized for 10 min. before use.

The primers C1-J-1754F, C1F356F, and C2-N-3661R (Integrated DNA Technologies, Inc., Coralville, IA) were used to amplify the COI/II mitochondrial genes (Simon et al. 1994, Degnan et al. 2004, Gómez-Valero 2008, Wernegreen et al. 2009). However, when the primers were used with the same methods in the referenced papers, they failed to produce adequate concentrations of PCR product. Instead, LCO-1490 and HCO-2198 primers were used to sequence 626 to 743bp long fragments of the COI mitochondrial gene (Supplementary material, Fig. 1). This resulted in a 267bp overlapping region with the COI/II fragments in Degnan et al. 2004. COI fragments were initially denatured with a Bio-Rad C1000 Touch$^{\text{TM}}$ Thermal Cycler (Bio-Rad, Hercules, CA) at 94°C for 2 min., then 35 cycles (94°C denaturation for 1 min, 45°C annealing for 1 min, 72°C extension for 2 min), then a final 72°C extension for 4 min. before going to a holding temperature of 4°C. 16S rDNA was amplified with Bloch16S-462F and Bloch16S-1299R primers (Degnan et al. 2004, Ramalho 2013, Wernegreen et al. 2009), which produced fragments from 763 to 824bp in length. 16S rDNA samples were denatured on the Bio-Rad C1000 TouchTM at 94°C for 2 min., then 30 cycles (95°C denaturation for 20 sec.0, 60°C annealing for 50 sec, 72°C extension for 90 sec), then a final 72°C extension for 5 min. before going to a holding temperature of 4°C.

DNA Yield Assays and Cleaning

Gel electrophoresis of all PCR products was run with VWR Agarose gel loading dye + GelRed (VWR Chemicals, LLC, Solon, OH) to confirm sufficient yield. Loading dye mix was prepared with 100µL 6X Loading dye, 500µL UV sterilized VWR pure.

Table 1: Camponotini collection localities and data, organized by subgenus

	Species	CUIC Database #	Collection Location	Latitude/Longitude	Accession/Collector #	Collector
Camponotus						
subgenus *Camponotus*	*Camponotus americanus*	—	Falmouth, MA, USA	—	AY334395, AY334379	Degnan et al. 2004
	Camponotus chromaiodes	—	Falmouth, MA, USA	—	AY334392, AY334376	Degnan et al. 2004
	Camponotus laevigatus	—	—	—	JN134908, GU226268	Schluns et al. 2011 (submitted)
	Camponotus laevissimus	—	Portal, AZ, USA	—	AY334384, AY334370	Degnan et al. 2004
	Camponotus modoc	CUIC000007039	Cloudcroft, NM, USA	32°56'28"N 105°44'13"W	SYW0419	Steven Wang
	Camponotus novaeboracensis	—	Falmouth, MA, USA	—	AY334394, AY334378	Degnan et al. 2004
	Camponotus pennsylvanicus	—	Concord, MA, USA	—	AY334391, AY196850	Degnan et al. 2004
	Camponotus schaefferi	—	Portal, AZ, USA	—	AY334388, AY334373	Degnan et al. 2004
	Camponotus texanus	CUIC000007040	Rumley, TX, USA	31°10'19"N 98°04'06"W	SYW0428,#ALW6281, UTIC ethanol 254719	Alex Wild
subgenus *Hypercolobopsis*	*Camponotus ulcerosus*	—	Portal, AZ, USA	—	AY334390, AY334375	Degnan et al. 2004
subgenus *Myrmaphaenus*	*Camponotus novogranadensis*	CUIC000007041	Sun Valley, FL, USA	26°29'47"N 80°12'44"W	SYW0420	Cole Icenhower
subgenus *Myrmentoma*	*Camponotus clarithorax*	—	—	—	JN134859, GU226295	Schluns et al. 2011 (submitted)
	Camponotus cuauhtemoc	CUIC000007042	Davis Mtns., TX, USA	30°39'56"N 104°01'45"W	SYW0413	Steven Wang
	Camponotus decipiens	CUIC000007043	Cypress, TX, USA	29°56'19"N 95°43'17"W	SYW0414	Steven Wang
	Camponotus essigi	CUIC000007044	Davis Mtns., TX, USA	30°41'47"N 104°04'57"W	SYW0415	Steven Wang
	Camponotus nearcticus	—	Falmouth, MA, USA	—	AY334396, AY334380	Degnan et al. 2004
	Camponotus sayi	—	Portal, AZ, USA	—	AY334385, AY334371	Degnan et al. 2004
	Camponotus snellingi	CUIC000007045	Monroe, LA, USA	32°32'55"N 92°09'56"W	SYW0425	Steven Wang
	Camponotus subbarbatus	CUIC000007046	Amity, PA, USA	40°28'39"N 75°16'00"W	SYW0427	Steven Wang
subgenus *Myrmobrachys*	*Camponotus mina*	CUIC000007047	Camp Creek, AZ, USA	33°52'09"N 111°44'32"W	SYW0418	Jake Nitta
	Camponotus planatus	CUIC000007048	Sarita, TX, USA	27°08'04"N 97°47'34"W	SYW0421	Steven Wang
subgenus *Myrmosphincta*	*Camponotus sexguttatus*	CUIC000007049	Sewall's Point, FL, USA	27°12'25"N 80°12'12"W	SYW0424	Cole Icenhower
subgenus *Myrmothrix*	*Camponotus atriceps*	CUIC000007050	Arroyo City, TX, USA	26°20'25"N 97°25'14"W	SYW0412	Steven Wang
	Camponotus floridanus	—	Gainesville, FL, USA	—	AY334397, AY334381	Degnan et al. 2004
subgenus *Tanaemyrmex*	*Camponotus castaneus*	—	Falmouth, MA, USA	—	AY334393, AY334377	Degnan et al. 2004
	Camponotus festinatus	—	Portal, AZ, USA	—	AY334386, AY196851	Degnan et al. 2004
	Camponotus fragilis	CUIC000007051, CUIC000007052	Scottsdale, AZ, USA	33°46'41"N 111°56'27"W	SYW0416	Jake Nitta
	Camponotus inaequalis	CUIC000007053	Sewall's Point, FL, USA	27°12'25"N 80°12'12"W	SYW0417	Cole Icenhower
	Camponotus ocreatus	—	Portal, AZ, USA	—	AY334387, AY334372	Degnan et al. 2004
	Camponotus pudorosus	CUIC000007054, CUIC000007055	Portal, AZ, USA	31°53'26"N 109°10'05"W	SYW0422	Tyler Boehmer
	Camponotus sansabeanus	—	Portal, AZ, USA	—	AY334382, AY334368	Degnan et al. 2004
	Camponotus semitestaceus	CUIC000007056	Hunter Creek, AZ, USA	34°18'36"N 111°02'18"W	SYW0423	Tyler Boehmer
	Camponotus socius	CUIC000007057	Paisley, FL, USA	28°59'02"N 81°33'29"W	SYW0426	Jonathan Ross
	Camponotus vafer	—	Portal, AZ, USA	—	AY334383, AY334369	Degnan et al. 2004
	Camponotus vicinus	—	Portal, AZ, USA	—	AY334389, AY334374	Degnan et al. 2004
	Camponotus zonatus	CUIC000007058	Little Havana, FL, USA	25°47'17"N 80°14'23"W	SYW0429	Queen coll. Armando Placeres, workers reared Aaron Stoll
Colobopsis	*Colobopsis etiolata*	CUIC000007059	Corpus Christi, TX, USA	27°40'03"N 97°19'40"W	SYW0433	Steven Wang
	Colobopsis impressa	CUIC000007060	Pensacola, FL, USA	30°26'35"N 87°19'37"W	SYW0430	Aaron Stoll
	Colobopsis mississippiensis	CUIC000007061	Sessums, MS, USA	33°23'33"N 88°42'42"W	SYW0431	Steven Wang
	Colobopsis obliqua	CUIC000007062	Pensacola, FL, USA	30°26'35"N 87°19'37"W	SYW0432	Aaron Stoll
	Colobopsis papago	—	—	—	JN134903, GU226272	Schluns et al. 2011 (submitted)

H_2O, and 150μL GelRed. Each agarose well received 3.0μL PCR product and 3.0μL loading dye mix. Electrophoreses were run in 1x TBE for 40 minutes at 80V and 200mA. An AMRESCO N550-300UL 100bp DNA ladder (VWR Chemicals, LLC, Solon, OH) was prepared alongside each gel to check approximate fragment lengths.

PCR products then underwent DNA purification through the ExoSAP-IT™ Protocol. 1.5μL ExoSAP was added per reaction and hydrolysis cleaning was performed in a Bio-Rad C1000 Touch™ Thermal Cycler at 35°C for 15 minutes, and then 80°C for 15 minutes.

DNA samples were then quantified through a Qubit 4 Fluorometer. 16S rDNA fragment yield was measured against BR STD1 and BR STD2 standards from Invitrogen (Thermo Fisher Scientific, Eugene, OR), and COI fragment yield was measured against HS STD1 and HS STD2 standards from Invitrogen.

DNA Sequencing

A BigDye DNA sequencing reaction was performed on the cleaned PCR products for each forward and reverse primer individually, totaling four separate primer batches of LCO-1490, HCO-2198, Bloch16S-462F, and Bloch16S-1299R. Each vial received 0.3μL Big Dye, 2.0μL 5x Buffer, 0.5μL 10 mM respective primer, 5.2μL pure H_2O, and 2.0μL cleaned PCR DNA. Sequencing reactions were performed in a Bio-Rad C1000 TouchTM Thermal Cycler, at 96°C for 1 minute, 25 cycles (96°C for 10 sec, 50°C for 5 sec, 60°C for 4 min). DNA samples were then submitted to the Genomics Facility at the Cornell Institute of Biotechnology for Ready-to-Load Sanger sequencing.

DNA Analysis

Forward and reverse sequences were assembled in Geneious (Kearse et al. 2012). The COI sequences after aligning and trimming were 267bp in length, the 16S rDNA sequences were 749bp in length. These were then aligned with MUSCLE 3.8.425 (Edgar 2004) with 8 maximum iterations. Alignments were visually checked in Mesquite 3.61 (Maddison & Maddison 2021), and extra spaces at each end were trimmed off. The fully aligned sequences were then run through a RAxML Maximum Likelihood analysis (Stamatakis 2014) as well as a MrBayes Bayesian analysis (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) on the CIPRES Portal (Miller et al. 2010). jModeltest 2.1.10 suggested TP3uf+I+G for COI and TVM+I+G for 16S rDNA. Since RAxML and MrBayes do not have these models implemented, the GTR+I+G model was used for among-site rate variation instead as an approximate equivalent. For Bayesian analysis, the number of substitution types was set at 6, using a 4X4 substitution model. Markov chain Monte Carlo (MCMC) was run for 10,000,000 generations in MrBayes, sampling every 1000 generations and discarding the first 25% as burn-in.

Co-diversification analysis was tested in Jane 4 (Conow et al. 2010) with the completed RAxML trees. For the genetic algorithm parameters, 100 generations with a population size of 1,000 were run in solve mode on the 39 host + 39 parasite tips for a total of 78 tips. In stats mode, 50 generations on a population size of 100 were run to generate a p-value distribution with the data, including original problem instances, with a sample size of 50, and random tip mapping. Two COI sequences from *Ca. decipiens* and *Co. impressa* failed to generate contigs, so they and their bacterial symbionts were excluded from the co-speciation analysis.

Results

The Sanger sequence contigs generated 626 to 743bp reads for *Camponotus* COI and 763 to 824bp reads for *Blochmannia* 16S rDNA. The total sequences represent 39 host species/endosymbiont associations total distributed across two host genera. Twenty novel COI and 16S rDNA associations were sequenced. All 8 *Camponotus* subgenera present in the continental USA are represented, including 4 species of *Colobopsis* (Table 1, Fig. 1). There were 22 total sequences for *Blochmannia*

16S rDNA, but 2 corresponding host COI contigs failed to align, so those 2 *Blochmannia* 16S rDNA were excluded from further analysis.

Outgroup Selection

Colobopsis was recovered to be sister to all Camponotini in Ward et al. 2016. Wernergreen et al. 2009 found that *Blochmannia obliqua* is also the earliest diverging lineage on their analysis across Camponotini, so the phylogenies were rooted on the node between *Camponotus* and *Colobopsis* to keep monophyly in both genera, with *Co. obliqua* and *B. obliqua* as sister to other USA *Colobopsis* and USA *Colobopsis* symbiont *Blochmannia* respectively. This is supported by *B. obliqua* being measured as the earliest diverging *Blochmannia* lineage in a previous Camponotini dataset (Williams & Wernegreen 2015).

Phylogenetic Modelling

In the *Blochmannia* 16S rDNA, the nucleotide frequencies were calculated with RAxML to be A: 0.283335, C: 0.196855, G: 0.290438, T: 0.229371. In the Camponotini COI mitochondrial DNA, the nucleotide frequencies were calculated to be A: 0.281277, C: 0.239281, G: 0.093596, T: 0.385846.

The reconstructed Bayesian and maximum likelihood trees are shown (Supplementary material, Fig 2), recovered with the GTR+I+G (INVGAMMA) model for sequence evolution (Tavaré 1986). The maximum likelihood trees were chosen for subsequent co-diversification analysis because they had no polytomies. Some of these generated trees have low support or polytomies on some nodes, which is likely due to the limitations of 16S rDNA of being a conserved gene (Poretsky et al. 2014) and may not show strong phylogenetic signal in some cases. Additionally, the limitations of the COI mitochondrial gene for phylogenetic signals must be recognized, including its ability to reach saturation quickly due to A+T richness (Morlais & Severson 2002). The incompleteness of the overlapping 267bp section of the COI mitochondrial gene sequenced also reduces available phylogenetic signal in the data.

Co-diversification Analysis

The Jane 4 (Conow et al. 2010) analysis of co-diversification found a significant p-value distribution from the original cost. Out of 69,064 solutions found, the most common isomorph which consisted of 61.6% of the total was chosen. Confidence values are shown on the nodes (Fig. 2). In statistics mode the original randomized cost was 37. With a sample size of 50, the observed cost had a median of 67 with a standard deviation of 2.18. The p-value cost histogram shows that the original randomized cost of no co-divergence does not overlap with the observed cost, which means that the p-value is significant and indicates possible congruence and co-diversification.

Discussion

The goal of this study was to test for co-diversification between US Camponotini and their *Blochmannia* endosymbionts, expanding on previous data. The purpose of this study is to generate new host and endosymbiont sequence data for USA Camponotini-*Blochmannia* relationships and use the larger dataset to test if co-diversification (Degnan et al. 2004) is still supported. This study generated 20 new Camponotini COI sequences and 22 new *Blochmannia* 16S rDNA sequences.

Most of the divergences that Jane 4 shows may result from the analysis picking up minor differences in the topologies of species divergences within a clade, since the *Blochmannia* does not tend to move out of an immediate clade. However, there is one such case where the *Blochmannia* not only jumps out of a clade but between subgenera. It was found that *Blochmannia novogranadensis* jumped between two unrelated subgenera, from *Myrmentoma* to *Myrmaphaenus*. Both the ML topology and the Bayesian bootstrap values showed high confidence in *B. novogranadensis* 16S rDNA within a group of *Myrmentoma*, in a clade with *B. snellingi*, *B. essigi*, and *B. cuauhtemoc*. In contrast, the COI mitochondrial sequences place *Ca. novogranadensis* outside of any *Myrmentoma* species. *Ca. novogranadensis* is introduced to Florida (Deyrup & Belmont 2013, Wetterer 2019)

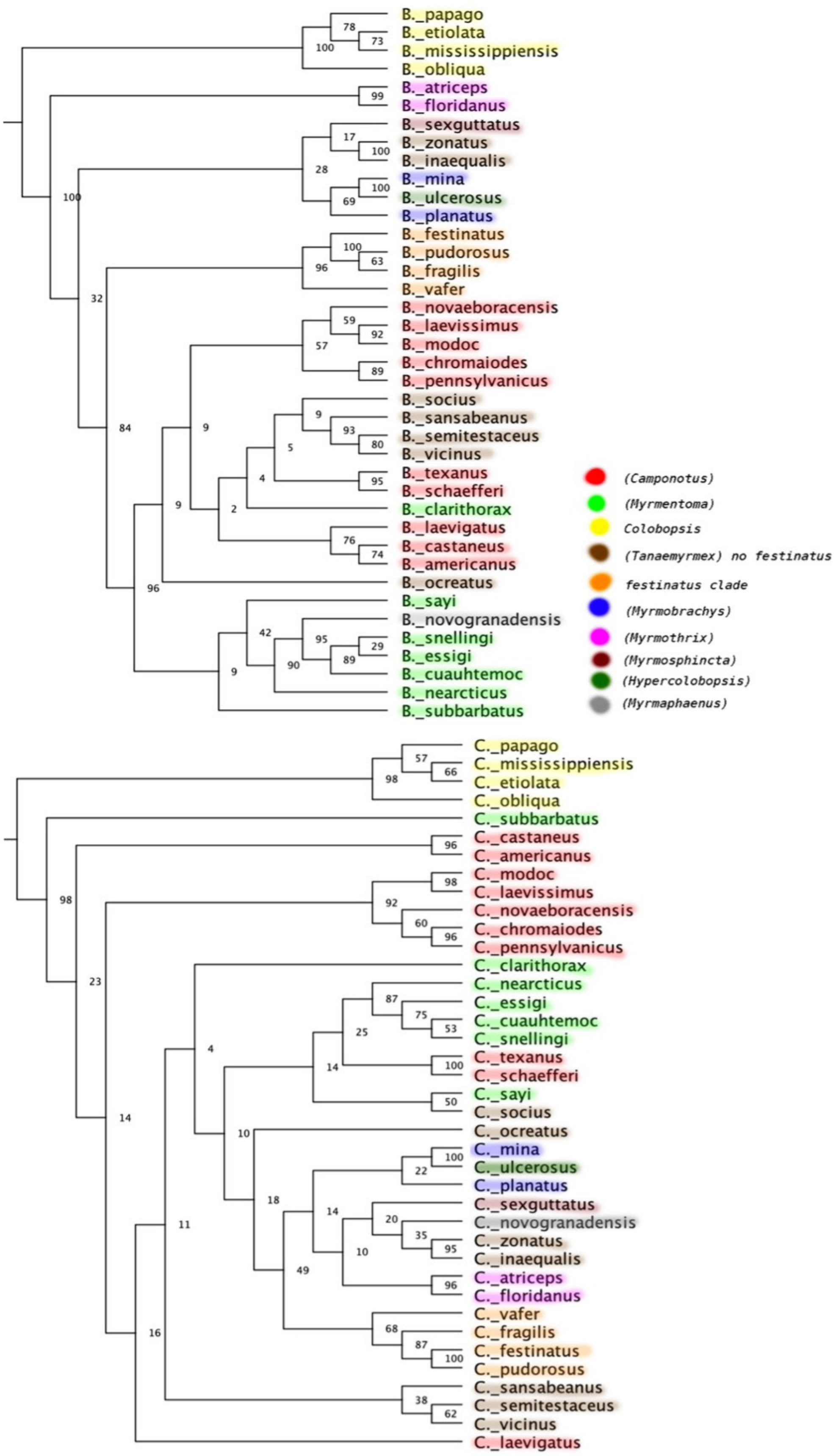

Fig. 1: Host genus and subgenus (in parenthesis) groupings mapped onto maximum likelihood cladograms of COI mitochondrial Camponotini and 16S rDNA *Blochmannia* trees. Calculated percent probabilities shown on nodes. Colors indicate subgenus groupings

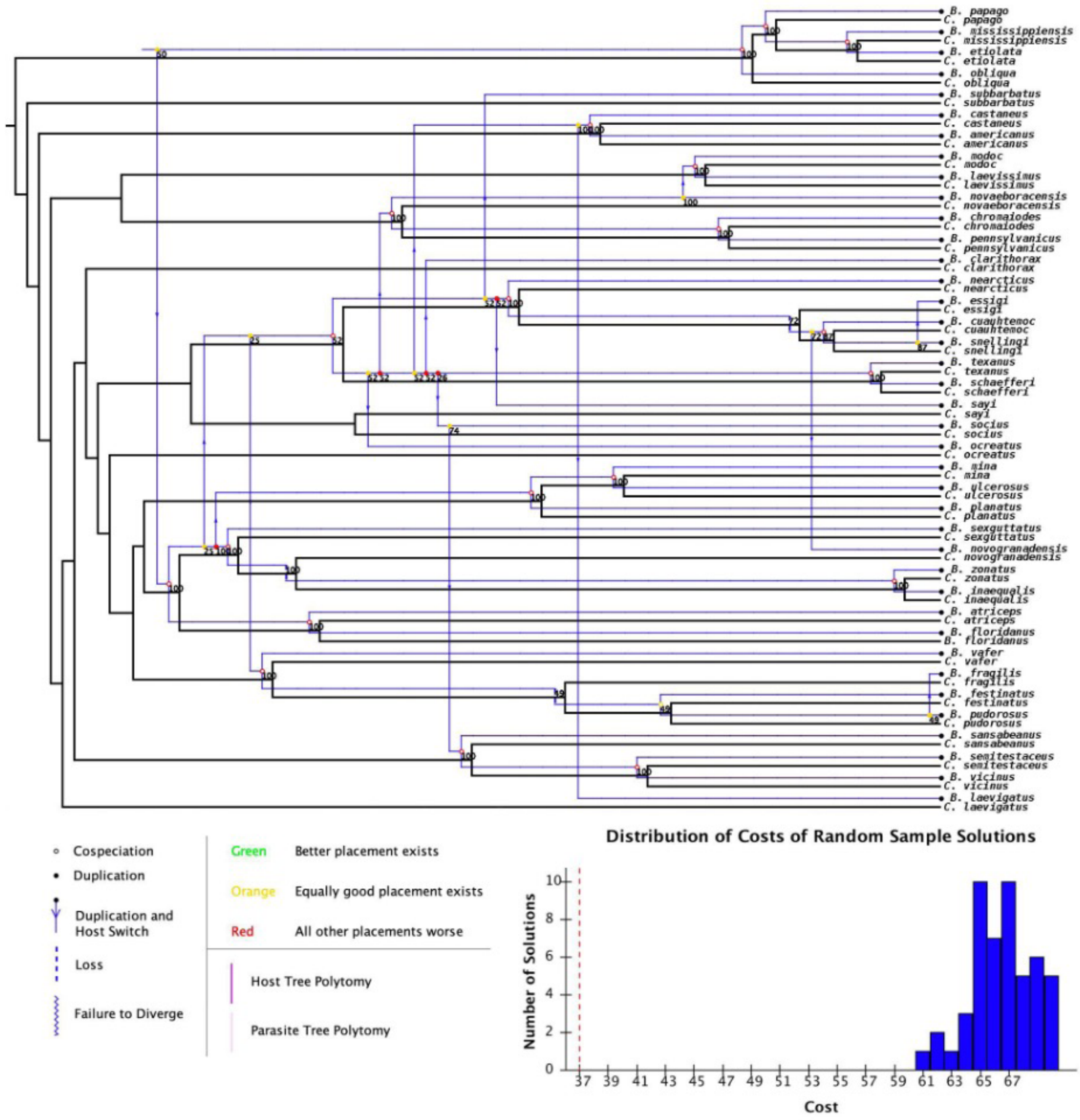

Fig. 2: Jane 4 co-diversification analysis. Original randomized cost is shown as a red dotted line on p-value cost histogram.

and lives arboreally, potentially in close contact with native *Myrmentoma* species. It is possible *Ca. novogranadensis* may have picked up this strain of *Blochmannia* from shared arboreal habitat, but more data is needed to conclude how and when this strain could have been horizontally transmitted.

Coverage of subgenera is increased in this study (Fig. 1), and this data supports many previous taxonomic groupings, like the *Ca.* (*Tanaemyrmex*) *festinatus* complex (Snelling 2006), the close relationship of *Ca. atriceps* and *Ca. floridanus* in the subgenus *Myrmothrix*, and the genus *Colobopsis*. It continues to give evidence that some broad subgeneric categories are inaccurate when grouping species of *Camponotus* by molecular phylogenetic relationships. The subgenera *Camponotus*, *Myrmentoma*, and *Tanaemyrmex* were recovered as polyphyletic, and *Ca. ulcerosus* could prove to be closely related to *Ca. mina* and not a true *Hypercolobopsis*. Like previous studies (Degnan et al. 2004), *Ca. schaefferi* arises from a group of *Myrmentoma* instead of from the nominate *Camponotus* subgenus, but in this study *Ca. texanus* comes sister to *Ca. schaefferi*, something hypothesized from COI mitochondrial data and morphology (Gadau et al. 1999, Mackay 2019). The recovered trees do not support *Camponotus laevigatus* (=past *quercicola*) as it is in the same clade as Ca. texanus and schaefferi (Gadau et al. 1999). In this study, *Ca. laevigatus* mitochondrial COI and its 16S rDNA *Blochmannia* symbiont were recovered within or close to a group of temperate nominate *Camponotus*, and never in a monophyletic clade with *Ca. texanus* and *schaefferi*.

Similar to previous studies on only *Camponotus* (Degnan et al. 2004), we have found evidence of co-diversification between *Camponotus/Colobopsis* and *Blochmannia*, which also used a GTR+I+G model for among-site rate variation and an ML phylogenetic tree. The co-diversification found in this study indicates a lack of horizontal gene transfer in *Blochmannia* (Degnan et al. 2004), like genome stability in the related bacterial endosymbionts *Buchnera* and *Wigglesworthia* (Akman et al. 2002 Funk et al. 2000, Tamas et al. 2002, Wernegreen & Moran 2001). Jane 4 codiversification analysis shows that *Blochmannia* have high fidelity of vertical transmission within a species of Camponotini and are tightly bound to their host species, but may be able to horizontally transmit, as observed for the first time in this study.

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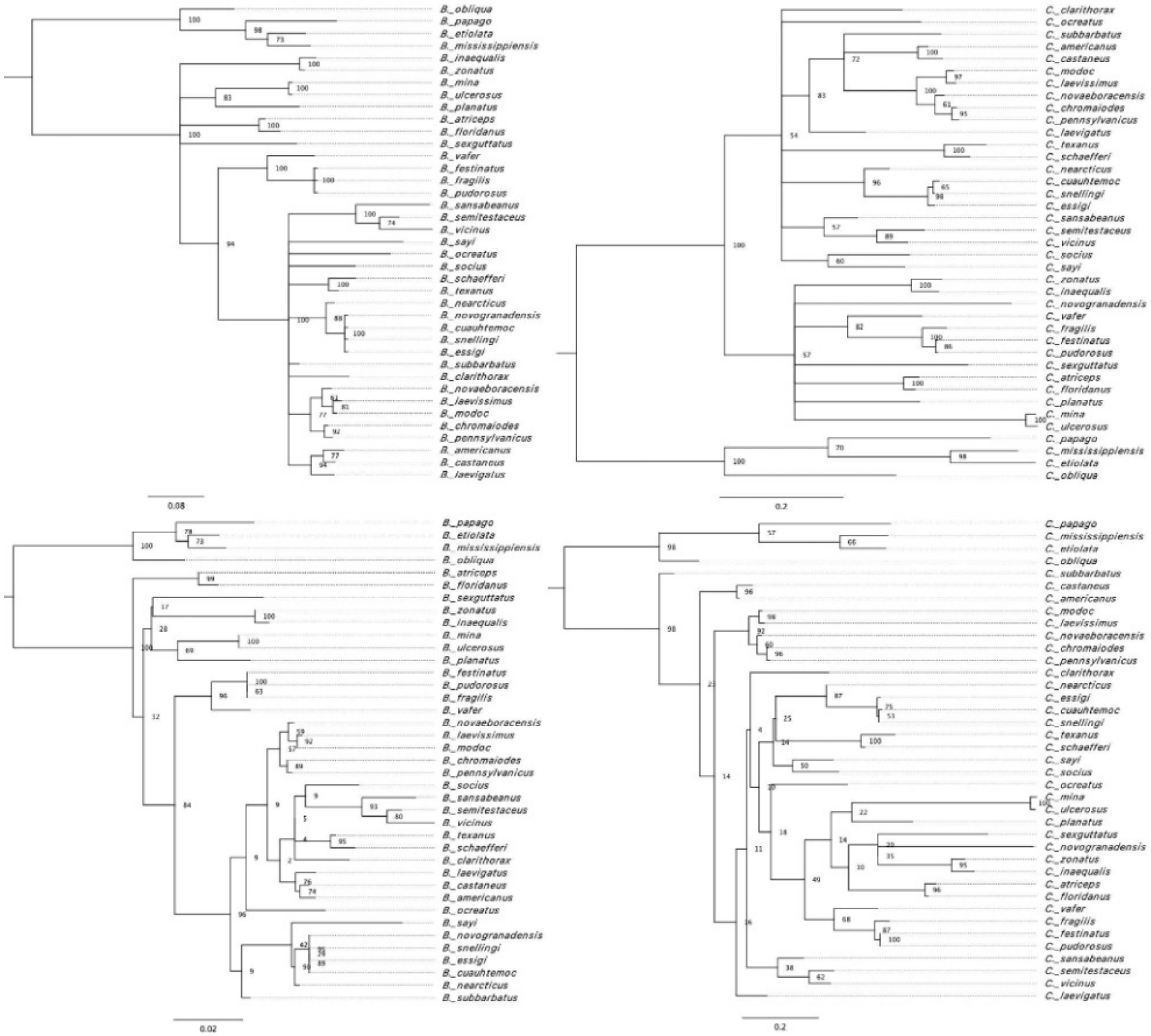
Supplementary Figures

COI/II	C1-J-1754Fb	CCACGTTTAAATAATATAAGATTTTGAC
	C2-N-3661Rb	CCACAAATTTCTGAACATTGACCA
	C1F356F	GGATCAGGAACAGGTTGAAC
	LCO1490	GGTCAACAAATCATAAAGATATTGG
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA
16S rDNA	Bloch16S_462F	AAACCCTGATGCAGCTATACCGTGTGTG
	Bloch16S-1299R	CCATTGTAGCACGTTTGTAGCCCTACTCA

Supplementary Fig. 1: Primer Sequences



Supplementary Fig. 2: Bayesian analysis (top) and maximum likelihood (bottom) endosymbiont 16S rDNA and host COI consensus trees, with genetic distances to scale and calculated percent probabilities.

Twitter Analysis of Pseudoarchaeology and Conspiracy Theories in Archaeology

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Abstract

General understanding of archaeology arises from mainstream news and entertainment alongside academic sources. However, there is increasing concern over the presence of pseudoarchaeology- claims that narrow history and are often based off circumstantial evidence. Online engagement can draw greater public support and attention for pseudoarchaeological claims. Those claims then act as fodder for supremacist ideas- often reattributing the accomplishments of ancient populations to the supernatural. In this paper, I approach Tweets about several archaeological conspiracies through network analysis. Network analyses facilitate broader study of discourse and the relationships between topics in a conversation, especially in online contexts. Analyzing the network structure provides a broad understanding of tweeted topics and how other online interests are connected to conspiracies and pseudoscientific material. By looking at the larger picture of archaeology in combination with specific Tweet examples, I characterize pseudoarchaeological engagement and affiliation on Twitter. I find that pseudoarchaeology related Tweets employ anti-institutional sentiment and draw on rhetorical themes in support of a historical white supremacy. This rhetoric breeds harmful notions about ancient and modern Indigenous populations while detracting from the goals of sincere archaeological research.

Keywords: archaeology, pseudoarchaeology, Twitter, disinformation, network analysis

Introduction

Misinformation and hoax news proliferate the modern public consciousness. For almost any current issue, from climate change to health policy, one is likely to encounter as much rumor and falsehood as fact-checked publication. This is also true for public engagement with archaeology. For as long as the past has fascinated us, history's unknowns attract interest in myths that lack archaeological basis and consensus from scholars. Some mysteries, like that of Atlantis, have sustained their intrigue long enough to generate whole narratives of their own. Though the enthrall of ancient conspiracies is not new, the methods used to disseminate them have changed dramatically. Popular media, like the famous *Ancient Aliens* TV show and discourse on social media have warped archaeological evidence to argue for unsubstantiated theories. *Chariots of the Gods* by Erich von Däniken not only inspires many of the episodes in *Ancient Aliens*, exemplifies the production of pseudoarchaeological content. In this paper, pseudoarchaeology is defined as claims that assert a very precise course of history, often supported by fallacy or weakly linking separate historical, geographical, and archaeological evidence. However, as this paper will demonstrate, these examples of pseudoarchaeology are not simply independent creations but stem from a longer history with roots in white supremacy and imperialism.

User behavior and the structure of social platforms drives the popularity of pseudoarchaeology and conspiracies. Social media today has strengthened the accessibility and scalability of networks, often uniting members through a common interest or goal. Those who push pseudoarchaeological claims have found audiences on spaces like

Twitter, where any amount of dubious information will go uncensored. These interactions can be observed through a network that encapsulates many users and tweets, painting a picture of the larger community engaged in archaeological or historical conspiracies. It also can tell us the range of topics that are connected to pseudoarchaeology or how a user may discover a claim by following linked accounts or hashtags. For instance, a user interested in #AncientAliens may look through that tag on Twitter and see another hashtag they want to explore, like #archaeology or #JoeRogan. By examining pseudoarchaeology discourse via the lens of Twitter networks and language, the breadth of is impact can be measured and potentially addressed. Background literature in pseudoarchaeology and the sociology of disinformation online informs this paper's analysis. From this research, I discuss how false or unsupported information associated with archaeology spreads, and what is distinct about the online language of pseudoarchaeology. Pseudoarchaeological speech on Twitter employs anti-institutional sentiment and draws on white supremacist rhetoric. Pseudoarchaeology and ancient conspiracies then engender harmful notions about Indigenous populations while detracting from the goals of sincere archaeological research.

Background

Pseudoarchaeology: Historical Background

It is true that claims within archaeology are not necessarily fact, and that there is not final confirmation as to what occurred in the ancient past. The frameworks used to produce archaeological knowledge continue to be challenged and re-written, most recently through feminist and decolonial lenses. Pseudoarchaeologists often blame elite academia for unjust exclusion of their work or censorship of "the truth" and frame their efforts as upending the status quo (Fagan, 2006). While these institutions do undervalue perspectives that do not originate from an established, often elite point of view, which has been rightfully critiqued, the work of pseudoarchaeology does not fit this scenario. To compare challenges to the frameworks of archaeology from scholars that do not represent the norm to that of pseudoarchaeology is a fallacy. Pseudoarchaeology does not broaden the space from which claims can emerge or further democratize the field, as pseudoarchaeologists would like to argue, but is instead a purposeful misrepresentation of the facts that arranges archaeological evidence into a narrowed view of the past (Fagan, 2006). One common theory posits that aliens were the true architects of the pyramids. To support this theory, *Ancient Aliens* relies on "striking" coincidences as its evidence, like the pyramids aligning with parts of constellations (Djokic & Thompson, 2017). This departs from both the historical and material record that support ancient humans who worked to plan, design, and construct the pyramids.

Pseudoarchaeology: Supremacist and Alt-Right Ideology

Steph Halmhofer (2021) writes about the connection between Ignatius Donnelly's writings about Atlantis and the Theosophical Society, founded by Helena Blavatsky, which began the spread of many Atlantis theories containing supremacist sentiment. A key facet of Blavatsky's *The Secret Doctrine* publication is that the superior "Aryan" race is descended from the Atlantean people. When conspiracists point to Atlantis as an idealized, highly advanced society, it speaks to a greater sentiment of white superiority over other peoples. It relays the idea that only those of white, European origin could possibly be responsible for the level of achievement ascribed to the population of Atlantis. It then appears unsurprising that a state that was chartered on white supremacy would also take an interest in the people of Atlantis: within Nazi Germany, officials were involved in researching Atlantis and finding evidence for Blavatsky's claims (Halmhofer 2021).

Several Atlantis or Atlantis adjacent theories position Atlanteans as a race that existed before native peoples, and often attribute parts of or all ancient society's accomplishments to this

Atlantis civilization. Halmhofer (2019) references a moment from a graphic novel that posits Atlanteans existing before Native Americans in North America. These notions, no matter their fictitiousness, can have serious impact on today's Indigenous communities descended from these societies. In the 19th century, American settlers believed in a lost race of white origin responsible for the country's large mound structures. It was believed that the Indigenous population encountered by colonists were incapable of achieving such a feat (Watkins, 2013). Native Americans were viewed as a barbaric people that had eliminated the previous, civilized white race. President Andrew Jackson utilized this myth in favor of Manifest Destiny, facilitating the Trail of Tears and backing the genocide of Native Americans (Zaitchik, 2018).

Pseudoarchaeology: Contemporary Dynamics

The digital age has further shifted how archaeology and pseudoarchaeology discourse are realized. Online spaces allow people to make connections based on common interest while transcending geographic divide or social background. Lorna Richardson (2013) notes the emergence of a "digital public archaeology" that is fostered by internet use. For archaeology in particular, the virtual world has the potential for increased public engagement from those with an interest in archaeology. Improved accessibility to archaeological findings online encourages a greater diversity of public participation in archaeological discourse. However, Richardson also observes limits to archaeological engagement, including skews in demographic and the restraints on platform content. The affordances of a social platform, like word count, the formats in which content can be shared, and exposure to other users, are just some factors in archaeology-related discussion online (2013). Many archaeologists run a blog or site that allows greater freedom to publish work or comment on other's pieces, but the Internet is far too vast for an average person to discover an archaeology blog as compared to following an account on Twitter or Instagram. The few places that are dedicated to archaeological findings among communities often become soundboards for pseudoarchaeological claims (Costopoulos 2018). Online users can easily turn to pseudoarchaeology, where its domain on the internet has greater reach.

Twitter and Network Analysis

Twitter creates a unique environment for sharing information, and has become an unlikely news source tool, like Facebook and other social media (Darius & Urquhart, 2021). Twitter's infrastructure thus becomes the determiner of how that news disseminates, from language choices motivated by character limits to trending topics driven by algorithms (Visentin et al., 2021). In recent years, concern has grown over the filtering of content based on political bias on Twitter and their impact on a user's political activity. Algorithms on social media may heighten cognitive biases because platforms have an interest in showing you related content to maintain high levels of engagement (Kitchens et al., 2020). A user is more likely to see relevant content on an account that is followed by the people they currently follow, and subsequently agree with that content. Twitter's algorithms and how users respond to the infrastructure of Twitter are relevant to the Tweets centered around conspiracy theories and how they reach their audiences on the platform.

Network analysis offers insight into the social and technological forces that may drive the proliferation of archaeological hypotheses. Such computationally driven analyses also increase the scale of possible study to look at larger patterns of online behavior as it relates to archaeology. In previous research, this has ranged from tracking networks of Twitter users from a scholarly community (Grandjean, 2016), political or social action movements (Jackson et al., 2020), to misinformation spread (Vosoughi et al., 2018). Zappavigna (2012) speaks to the use of social media as its own dataset within linguistics for analyzing the unique features of social media language. Tweets are "microposts" that share many of the personal features of a blog but are limited by a short character count. In addition to mining a text corpus from Tweets, data from

Twitter can also contain relationships between posts and users.

Social networks such as Twitter have especially pushed forward research within network analysis. D'Andrea, Ferri, and Grifoni (2010) outline the history of methods used with social networks and the growth of the field. Because of the diversity of uses for social media, many kinds of relationships can be studied over a wide range of content. The online social network subsequently has several definitions based in as many perspectives, including the sociological, technological, and economic. The "socio-centric" category of social network analysis focuses on relationships among the nodes (points) of a network as "social collectives" as opposed to the "ego-centric" strategy of looking at connections to a specific actor or set of actors in a network (2010). The socio-centric method of analysis builds this paper's study to help understand the overall behaviors within pseudoarchaeology related Tweets and they ways they are connected via hashtags. Hashtags also go beyond a single user or users and instead brings many Tweets together under one category. Through this, I analyze comprehensive discussion topics, rather than the interactions of a few pseudoarchaeology accounts.

Methodology

The resulting networks represent three hashtags: #ancientaliens, #ancientastronaut, and #atlantis, all related to pseudoarchaeological claims and conspiracy theories. The hashtags #ancientaliens and #ancientastronaut focus on the TV show *Ancient Aliens*. Atlantis refers to the myth first featured in one of Plato's works and is popularly known now as a lost, underwater city with an advanced society. These tags refer to some of the most popular conspiracies associated with archaeology as well as being mainstream concepts. Each of these topics is largely accessible to an individual without archaeological education or interest in other conspiracies. Not only would the hashtag serve as discussion point in a pseudoarchaeology Tweet, but it can also be how a user may discover more about archaeology online. To limit the amount of data and set a specific date range, Tweets collected were limited to the past decade running from 2012 to 2022.

By looking at a network of hashtags related to archaeological conspiracies we can get a big picture representation of its subtopics and the ways those topics intertwine with each other. Hashtags are used in promotional aspect since users can use hashtags to search for Tweets, helping to signal the target topic of this paper. Many Tweets will use multiple hashtags, often because they are of a similar subject or the user who created the Tweet wants to connect their post to other topics, which can be represented a link between two hashtags. In these networks, there is an "ego" hashtag node to which all other hashtags are connected. For example, we can collect Tweets with the #ancientaliens tag and subsequently catalog all the other hashtags that appear in those Tweets. An edge (link) will exist between two hashtags if they appear in a Tweet together. So other hashtags in the #ancientaliens network will connect to that node, but we can also see relationships among the subtopics of #ancientaliens. Colored groupings are determined by greater connectivity with other nodes, meaning that two hashtags that share the same color are more likely to appear in Tweets together compared to different colored tags. The sizing of nodes indicates degree, meaning the number of edges towards the node. For instance, #ancientaliens appears the largest in its own network since it appears in all the Tweets.

By getting an idea of the smaller segments within conspiracy theories, we can understand more about the potential motives, sentiments, and rhetorical strategies of Tweets within a pseudoarchaeology network. To create such a network, I collected Tweets containing the hashtag of reference using a Twarc command. This is a tool used in the computer's command line to connect a user to the Twitter API. Twarc has specified commands that can harvest Tweets and their metadata, along with account information and other user data. As part of the data preprocessing, Twarc also allows for a time frame and maximum number of Tweets to be collected, helping filter down to relevant Tweets. The collected nodes and connections can then be visualized on software programs like Gephi. For this data collection, the data collected is purposely limited to pseudoarchaeology hashtags, but there are additional biases based on the demographics of users likely to be on Twitter as well as platform exposure to the hashtags.

Gephi is a free software that allows users to create new network data in a Gephi file or upload already created data. For the graphs in this paper, a few major features of Gephi were utilized. First, many of the hashtags in the original data were filtered out using the Giant Component and Degree Range filters. These allow the user to focus on the largest connected component of the graph and filter out hashtag nodes that are less popular. The term "degree" for these networks refers to the number of connections a node has- in this case how often it appeared in Tweets with the target hashtag. This was key for focusing on the hashtags with the most usage. This aspect of degree was also utilized to select the sizing of the nodes. Nodes that are highly connected in the network become larger, so the target hashtag becomes the largest sized. Lastly, a Modularity Class algorithm was run on the nodes to create rough color organization. This algorithm calculates a given number of "communities" in a network based on what hashtags most often appear in Tweets together. Hashtags that appear together can convey a connection amongst subjects, current news, or a user's own interest. If Tweets are organized into a community together, they receive the same color. Using Gephi, I've outputted several network images for this paper.

Results

The network graphs of #ancientaliens and #ancientastronaut summarize an online discussion closely linked to space, aliens, and UFOs, while bringing in common topics from history or archaeology. Both networks also include subjects from alien conspiracies and reference popular sources of pseudoarchaeology, pointing to an online sphere that frequently finds connection between the ancient world and the extraterrestrial. They also expose links to sources that connect back to the ideologies of the alt-right or are consistent with supremacist content. The network of tags for #Atlantis, in comparison, is less narrow. A range of ideas follows Atlantis, touching on fictional renditions or even travel destinations. It also reveals the variety of conspiracy theories about Atlantis, including alien gods and advanced societies. Overall, the pseudoarchaeological world of Twitter is one that consistently associates the efforts of ancient humans and archaeological research with the supernatural, forming a view of the world that devalues indigenous existence and academic findings.

Ancient Aliens on Twitter

In the decade following 2012, the term "ancient aliens" has been tweeted or retweeted more than 1.1 million times. The use of ancient aliens in Tweets has steadily increased since 2017 and peaked at the start of the COVID-19 pandemic, based on frequency of mentions. Findings presented on the *Ancient Aliens* show, like the theory of an asteroid that wiped out the societies of ancient North America or another that claims the Earth is hollow, have also seen increases in engagement on Twitter, though with significantly fewer Tweets than the broader category of ancient aliens. Along with the fervent fans of ancient alien theories, skeptics of such historical claims also have a Twitter presence. Terms like "pseudoarchaeology" and "pseudohistory" rapidly increase in Tweet mentions after 2017 and 2018.

The hashtag #ancientaliens and the hashtags featured in the same Tweets indicate topics related to the TV show or discourse around alien conspiracies. Observation of its hashtag network showcases these relationships (Figure 1 below). Some of the most popular companion hashtags are #aliens, #ufo, and #space. Other hashtags reference the past, like #ancientEgypt and #archaeology. A few hashtags reference the media that discusses pseudoarchaeology, like the Erich Von Daniken book *Chariots of the Gods*, Joe Rogan's radio show, and spiritual site Gaia. The hashtag #ancientastronaut also works as a signal

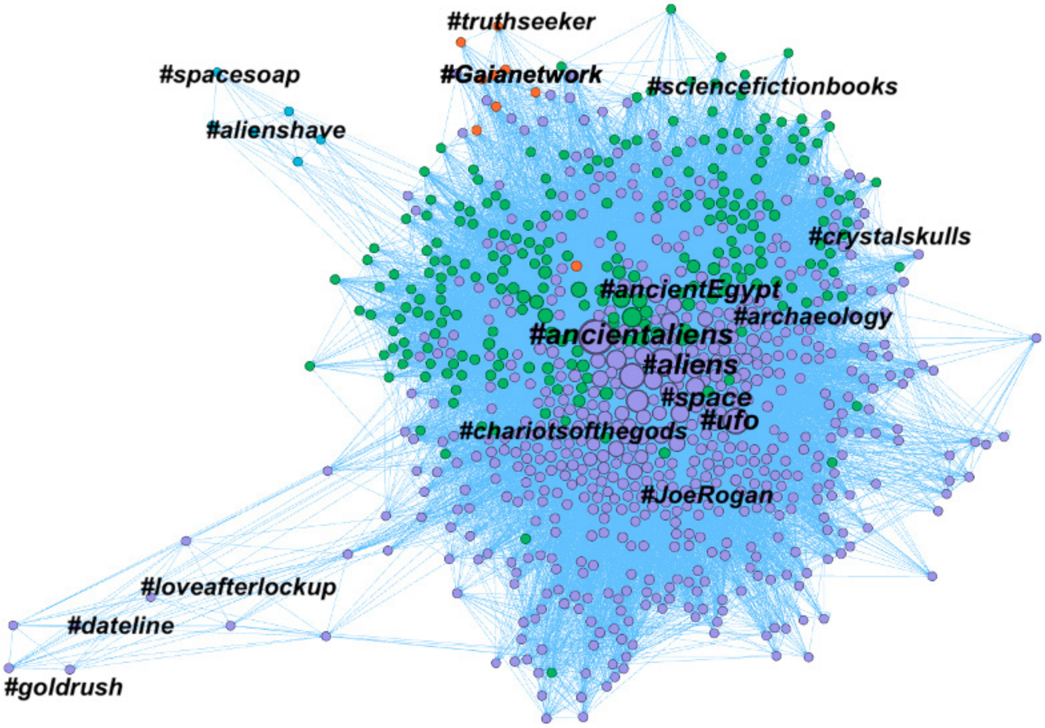

Figure 1: Network representing hashtags that appear in Tweets using #ancientaliens.

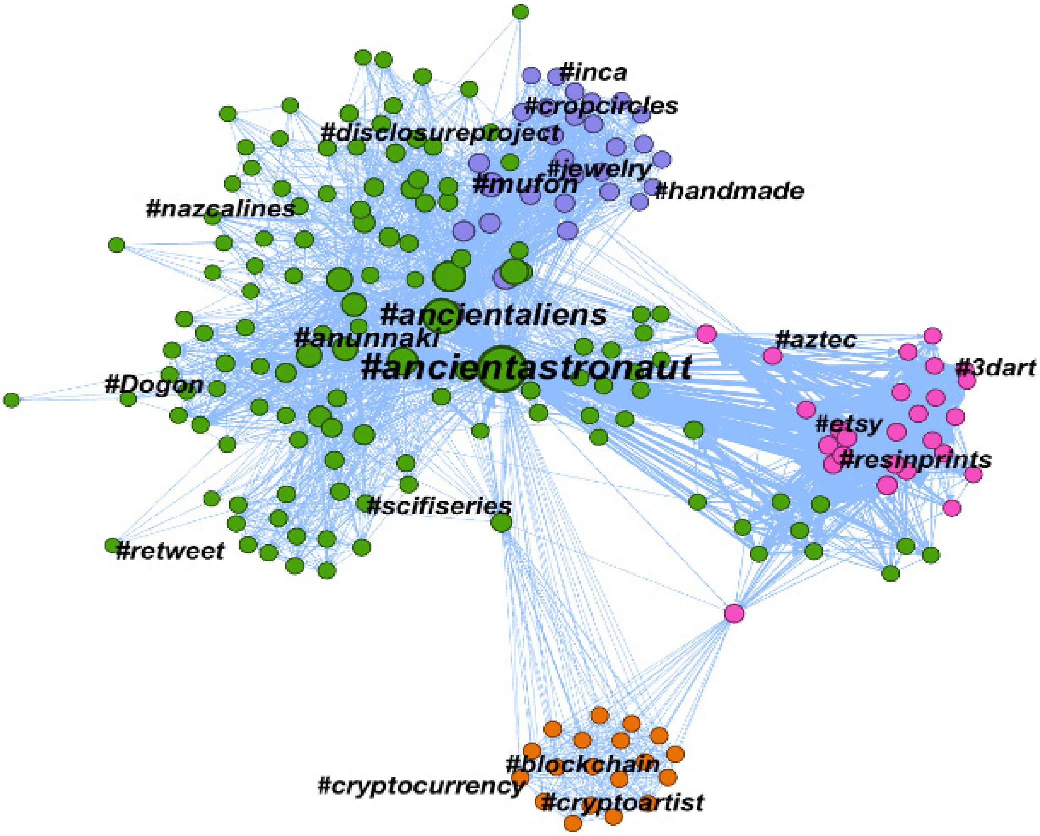

Figure 2: Network representing hashtags that appear in Tweets using #ancientastronaut.

regarding conversation about belief in aliens influencing the past, along with other claims in supernatural history. Since it is not as explicitly tied to the *Ancient Aliens* show, fewer Tweets are promotional of the show or tied to its production.

The network graph for #ancientastronaut (Figure 2 above) again illustrates relationships between topics similar to #ancientaliens. The network's colors provide a starting point to organizing the topics within #ancientastronaut. The green nodes constitute a majority of the graph and do not have an obvious category compared to smaller, more precise groupings. Several of the hashtags allude to archaeological sites, artifacts, or terms, like the Peru's Nazca Lines or the indigenous Dogon people in Mali. The light purple nodes are also a looser grouping and include a few different broad categories of hashtags. These are #alienlife and #cropcircles, or some that appear more distant, like #jewelry and #handmade. Other craft-related hashtags appear more strongly in the nodes grouped in pink, which also include #resinprints, #etsy, and #3dart. The orange cluster consists of hashtags like #blockchain, #cryptocurrency, and #cryptoartist. Many of the orange hashtags originate from a single Twitter account. Since the user will use the same grouping of hashtags, they will produce many instances of the hashtags appearing together in a Tweet, thus placing them into the same color category.

Some archaeology conspiracy and ancient history related hashtags are also connected to one another within the #ancientastronaut network. #Annunaki also appears in Tweets with the hashtags #Maya, #Ziggurat, #Nibiru, and #Nephilims. Annunaki, or sometimes spelled Anunnaki, refer to Babylonian gods, while the Nephilim are a race of people from the Bible. Both have been appropriated to refer to ancient extraterrestrial beings treated as gods (Flaherty, 2011). Several theories about the Annunaki circulate through Tweets or tweeted blog posts, like one linking to a post about the "Igigi," another set of aliens rebelling against the Anunnaki extraterrestrials, who apparently created humans to serve as laborers (UFO- AC Research Group, 2020). As their evidence, they reference texts like the Epic of Gilgamesh, or use images of Mesopotamian reliefs that appear to depict their hypotheses. Many of these originate from New Age religions that seek out evidence of alien influence on Earth through biblical and other ancient texts. Nibiru, another hashtag, was thought to be the Babylonian name for Jupiter, but is the apparent home planet of the Nephilim (Flaherty, 2011). The ziggurat, a type of temple with levels of platforms, is a notable example of Mesopotamian architecture (Seymour, 2011). On Twitter, conspiracists use them as evidence of the alien Annunaki's achievements, even using doctored photos of a similar looking structure on the planet Mars (Twitter User 1 2021).

Tweets related to ancient societies use a variety of visual aids to enrich their claims. One Tweet uses pictures of Mesoamerican artifacts, interpreted to have alien-like features, with some clearly faked to showcase stereotypical sci-fi alien anatomy. The Tweet author calls the images "incredible discoveries," remarking that they "havent seen these in the news" as well as tagging #QAnon and #DeepState (Twitter User 2 2019). A few Tweets also exhibit what the authors find to be striking similarities across cultures, both past and present. One compares reliefs of Mesopotamian hairstyling with the curled wigs worn by British parliament as well as a sphinx carving with an image of a sphinx on a present-day church signboard (Twitter User 3 2019). Another tweeted out images comparing instances of long-horned, often female figures, spanning Spanish cave drawings to a type of Mongolian national dress (Twitter User 4 2022). Both Tweets connect various cultures and time periods by jumping to a supernatural explanation, without considering the sociological reasons for how similar customs can originate in different places. Such Tweets often lack direct statements about the connection between alien and human history, but instead imply their intention by tagging #ancientastronaut, letting the hashtag add additional meaning to their post. Since these come from accounts heavily invested in conspiracy theories or alien evidence, they imply the larger goal of proving ancient alien existence.

The idea that aliens are kept hidden by the government frequently appears on Twitter. A rather prolific example shows an image of Trump and states "I would love the one good thing #DiaperDon ever did in office to be revealing massive historic #UFOCoverUps by USA" (Twitter User 5 2020). The hashtags #stevengreer and #disclosureproject also appear in conjunction with #ancientaliens. A recent February Tweet questioned the removal of the Giorgio Tsoukalos and UFO-enthusiast Steven Greer episodes from Joe Rogan's Spotify podcast, wondering why aliens would deserve censorship (Twitter User 6 2022). Greer's claims include that several public figures, including President Kennedy, were killed for sharing information about extraterrestrials, and that alien technology retrieved from UFO's motivated innovations in human invention (Lewis-Kraus, 2021). Several Tweets using the #disclosureproject tag attach videos of blurry proof of alien UFOs, and feature #mufon, which stands for Mutual UFO Network. Like #Annunaki and #Ziggurat, hashtags related MUFON or alien cover-ups draw readers into a rabbit hole of conspiracies that seemingly connect the dots through human history. This is another example of how pseudoarchaeology does not follow a typical evidentiary argument, but instead tries to convince the reader that such coincidences in history are not possible, thus aliens or other supernatural forces must be at play. to convince the reader that such coincidences in history are not possible, thus aliens or other supernatural forces must be at play.

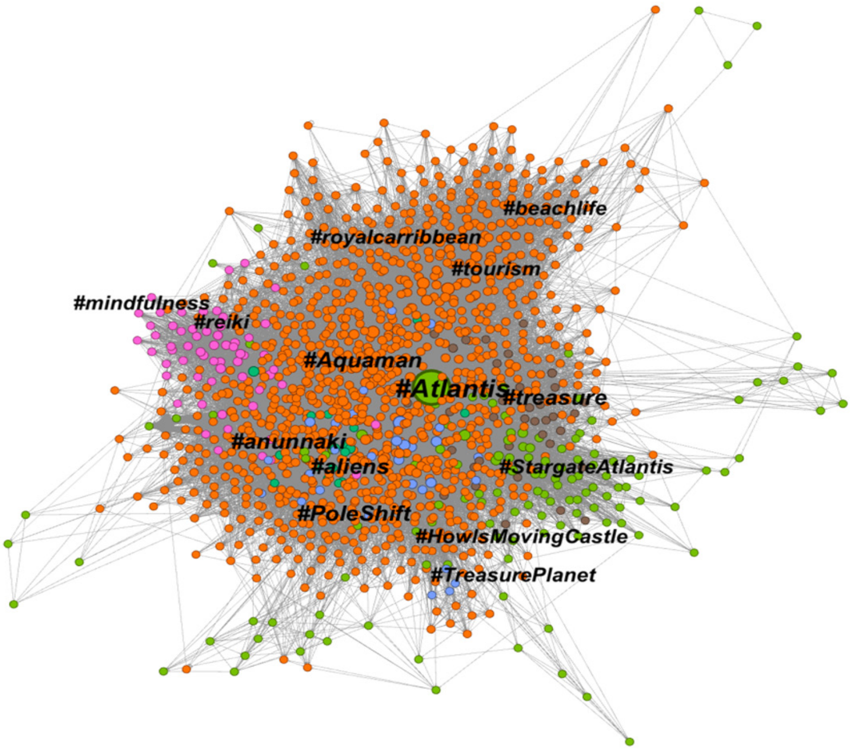

Figure 3: Network representing hashtags that appear in Tweets using #Atlantis.

Atlantis on Twitter

In the Atlantis hashtag network (Figure 4), there are several unexpected subtopics, like animated movies, spirituality and meditation, and tourism. The 2001 film Atlantis: The Lost Empire is likely connected to Tweets related to animation or Disney, including #TreasurePlanet and #HowlsMovingCastle. There's also a set of hashtags that include #mindfulness and #reiki, which are terms from a modern spiritualism associated with meditation and crystals. The website Gaia is well known for this strain of spiritualism, and features in a hashtag from the #ancientaaliens network. Some Tweets features #larimar, a type of vibrant blue stone used in jewelry that Twitter users associate with Atlantis (Twitter User 7 2020). #Aura and #Reiki accompanies various "Atlantean gemstone" advertisements, used by accounts to publicize the crystals or crystal creations they sell. One Tweet even alleges that Atlantis people used quartz for "communication devices" and "sources of energy" (Twitter User 8 2022). In this case, the advertising pull of Atlantis lies with the notion of Atlanteans being extremely advanced and stewards of an ancient knowledge. Crystal healing and parts of spiritualism are known for their rejection of the institutional, often branded alternative medicine. Much of this discussion around healing, spiritual beliefs, and the work of aliens comes together on the Gaia website (Gaia, Inc, 2022).

Accounts that aim to give proof of Atlantis's existence often refer to maps that show its potential location, as well as locations of related "lost civilizations" like Lemuria or Mu. One tweeted map places Atlantis as a large land mass in the Atlantic and Lemuria as another giant one that spans the Pacific Ocean, accompanied by hashtags like #WorldMap and #Truth (Twitter User 9 2021). As in many Tweets, their sources for this information are unclear or nonexistent. One user posted what appears to be screenshots from an interview about aliens and their connection to earth history, claiming that not only did Atlantis exist, but that it was an alien creation (Twitter User 10 2022). Alongside the many outlandish statements, one user notes the connection between Atlantis and Charles Brasseur de Bourbourg, who helped inspire Ignatius Donnelly's writings on the legendary city and facilitated a growing mythology around lost societies (Twitter User 11 2022, Halmhofer 2021). Even to the present day, discussion of Atlantis is linked to authors of supremacist ideas, promoting the features of the Atlantis myth rooted in proving the existence of an "advanced race." Some of the ideas featured in #ancientastronaut and #ancientaliens also appear in this network, like #anunnaki, #StargateAtlantis, and #PoleShift, which is a reference to one of the *Ancient Aliens* theories about catastrophic natural disasters due to shifts in Earth's poles.

Discussion

Pseudoarchaeology as Disinformation

The growth of engagement with conspiracy and pseudoarchaeology online coincides with a reworking of the Information Age. Andrew Marantz (2019) discusses in his book *Anti-Social* the rising role of Twitter and viral social media posts in politics, particularly in the 2016 election. Marantz identifies a growing community of anti-establishment Twitter influencers aligned with the alt-right movement. What Marantz sees as key to their political capability is not just that they often support populist and nationalistic viewpoints, but that they created posts that are purposefully vitriolic to draw attention and a following. Marantz's concerns with their use of social media posts are also echoed among conspiracist Twitter users. As Marantz argues, they cannot be granted political legitimacy because they reject dialogue. This is the danger of pseudoarchaeology and its growth in these spaces- its disseminators refuse to consider the potential of other solutions in understanding the vibrance of human history, often with a purpose to invoke distrust of authority. They sow further rejection of "mainstream media" or institutional sources, like government agencies, or in this case, the world of academia.

A considerable element of many Tweets that try to prove alien presence, existence of Atlantis, or other lost civilization hypotheses, would be the use of visual material. Whether that be in a map, selected artifacts, or even self-created art depicting their claims, visuals can affirm their Tweet or add more information about what the Twitter user would like to imply. Some images are real elements of archaeological sites, and yet conspiracists come to vastly different conclusions about them. One of the clearest examples is Erich von Daniken's analysis of the tomb of Pakal in Palenque, associated with ancient Maya culture. Often regurgitated in ancient alien Tweets, Daniken concluded that the tomb carving represented an ancient astronaut (Evans, 2012). We can see similarities between alien or Atlantis conspiracists with Tweets from the alt-right, as both try to vilify establishment science or perceived authority over subject matter, while also fabricating supporting evidence meant to seem obvious to the Tweet's reader.

Several of this paper's hashtags appear unrelated to archaeological or extraterrestrial fields but instead share the sentiment of going against the mainstream or fighting back against a perceived establishment. Cryptocurrency, and other topics related cryptocurrency's technology, like #blockchain and #nft, are also present in the hashtag networks. Cryptocurrency is known for being an unregulated currency, with limited attachment to governments or a central institution. It is no surprise that figures in supremacist movement invested in crypto in its early stages, then capitalized on it to fund campaigns outside of government purview (Hayden & Squire, 2021). Several hashtags also reference the site Gaia or terms related to spiritualism. On Gaia's own website they tout their many web series about "topics you won't find in the mainstream medium" (Gaia, Inc, 2022). When people enter the Twitter space around *Ancient Aliens* or the Atlantis myth, they consistently encounter a worldview that vilifies established institutions.

Impact on Indigenous Communities

Credit to achievement in history matters. Antiquities that are perceived as accomplished are assigned a high degree of intelligence, craftsmanship, and civility, often tied to connotations of the labels "primitive" versus "advanced or complex." That perception confers esteem to the modern population regarded as closest to them (whether or not that should be the case). The structures around approaches to science and politics in the West today were specifically chosen out of an admiration for the Greek and Roman democracies and republics. But for the peoples that do not belong to this euro-centric perspective, their history and current ontologies are consequently othered. European and white histories act as the basis upon which other cultures are compared, centering white perspectives over others. For North American native groups, this has led to misrepresentation in museums and a lack of respect for the Indigenous past, causing greater susceptibility to cultural appropriation and removed agency over artifacts and ancestral remains. It also seeps into broader disputes over land and self-governance for modern native groups. Indigenous knowledge and ontologies are considered secondary to the scientific approaches that originate from societies viewed as white and European. One alt-right blogger accuses the Ancient Aliens show of attributing Peruvian cranial remains to aliens when it should instead be the white race praised for establishing complex structures and states across the globe (Chouinard, 2017). Both the show and site author, of course, completely disregard Peruvian indigenous cultures, yet while recognizing that history bestows status in the modern world and is necessary to formulate supremacist arguments.

Perception of Archaeology on Twitter

Archaeology can already find depictions in classic media that lack the nuance and a full picture of a site's archaeological evidence. Social media can even more easily warp archaeology news and exposes such misinformation to a larger audience. The abundance of sources on social media means those entities must vie for the reader's attention. The need to appeal to an audience has allowed fake news to capitalize on an emotional response from consumers (Giusti & Piras, 2020). Posts with false information or conspiracy theories then increase in popularity. Increasing regulation from social media platforms comes with little recompense. The more a userbase is attracted to a platform and stays on the app the more lucrative advertisement spots can be. Platforms are then reluctant to over-police their communities, with Twitter being the most notorious for this. For many years, Twitter famously did not ban hate speech from its platform until around 2016, causing several incidents involving gender and race-based harassment (Jeong, 2016).

Archaeologists need to be aware of the growing presence of pseudoarchaeology on these platforms lest they further develop into the main perception of archaeological work rather than its outlier. Drastic consequences occur if "alternative" histories are left unchecked. Russia famously interfered in the 2016 U.S. election by strategically dispersing large amounts of fake content. It was not simply to persuade consumers of a theory or claim, but instead facilitate widespread distrust in the institutional (Brantly, 2020). When Twitter users come across hashtags about ancient aliens, much of the content misrepresents or presents false archaeological evidence. When much of the public's exposure to archaeology are shows like *Ancient Aliens* or mythical places like Atlantis, their access to the field begins with misleading evidence and conspiracies that invalidate histories of real people. Popular media such as these is generally more accessible than discussions that are almost exclusively held in academic spaces. It is often the exclusive nature of academia that makes *Ancient Aliens* or conspiracy threads more appealing, even exciting, to their consumers. Once on Twitter, tags like #ancientaliens open a rabbit hole of connected theories that promote a pseudoarchaeological worldview. With Tweets that seek to subvert academic sources or institutionalized science, academia is viewed as either a gatekeeper of conspiracy evidence or elitists that staunchly reject any opposing view.

Conclusion

The issue with conspiracies in archaeology is not that they potentially pose risk to the hardline knowns of the past and our perception of "fact," but they situate themselves into a far stricter line of reasoning than they accuse current science of instituting. Their arguments often rely on a single identified pattern, like matching shapes in ancient monuments or contrived star alignments, when the reality of the world is far richer. Pseudoscientific arguments consider only a single perspective from the material record out of the many possibilities about the past. Network analysis of pseudoarchaeology on Twitter uncovers this web that surrounds popular conspiracies and demonstrable how reachable it is from mainstream media. Exposure to the notion that archaeology and our understanding of history is dynamic and draws on many frameworks can help subvert the inclination towards conspiracy or pseudoarchaeology. Archaeological academia must increase their participation on social media and more deeply investigate how it impacts archaeological perception. Archaeology will inevitably be discussed online, so having more archaeologists that add their own posts can start building a community with the standards that we seek for discussion. By approaching pseudoarchaeological arguments with a worldview that looks beyond one alignment of dots, a far more interesting universe of constellations can be revealed.

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Phenotypic Variation in Introduced Rock Pigeons (*Columba livia*) In the Eastern United States

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Abstract

As an introduced species in North America, the Rock Pigeon (*Columba livia*) has established itself in a vast array of climates and niches. Rock Pigeons exhibit great phenotypic variation in their plumage in captivity and in the wild. In the time since the introduction of *C. livia* to North America, this phenotypic variation in feral pigeons established in different regions may have allowed them to adapt to local environmental conditions. To explore this idea, I focused on analyses of plumage and morphological phenotypes in feral Rock Pigeons across the eastern United States of America. I found that northern Rock Pigeon populations had a heavier body mass, longer wing chord, and longer head length than their southern counterparts. This geographic variation in phenotypes observed in feral Rock Pigeons in the eastern United States suggests this species may be poised to respond to local selective pressures.

KEYWORDS: Rock pigeon, *Columba livia*, microevolution, North America

Introduction

Introduced species offer a unique opportunity to understand how quickly populations can change in morphology, behavior, and other aspects of life-history (Johnston and Selander, 1964 Hughey et al. Gilchrist et.al, 2001,Chun et.al, 2007, Hendry, 2008). If an introduced species successfully colonizes a new location and then subsequently expands its range, these species should respond to differences in environmental conditions across their new range, especially if conditions differ from their native ranges. Studies of introduced species have found rapid phenotypic changes across the introduced geographic ranges, but these studies often focus on less mobile species (plants, fruit flies and lizards). Here, I examine for similar phenotypic changes across geography in a mobile bird, the feral Rock Pigeon (*Columba livia*).

Previous work on the near globally-ubiquitous House sparrow (Passer domesticus) has demonstrated significant size variation in its introduced ranges. Introduced populations in North America show greater body length, wing length, and bill length than their European counterparts. Moreover, within the introduced North American populations, significant morphological variation exists. Specimens from Death Valley California had the palest plumage coloration, while specimens from Mexico City had the overall darkest coloration. Similarly, regional variation in body mass showed the heaviest individuals in Quebec, Canada with average masses of ~31 grams, and the lightest individuals in Oaxaca, Mexico with average masses of ~27 grams (Johnston and Selander, 1964). These patterns are consistent with predictions generated by Bergmann's Rule (Ashton, 2008), where the heaviest individuals are furthest north. House sparrows were introduced into North America in 1851, giving them a span of 133 years between their introduction and Johnston and Selander's study to diverge.

Johnston and Selander's (1964) study of introduced House Sparrows suggests that other introduced bird species might show similar levels of phenotypic variation across their introduced ranges. I examine for similar patterns of geographic variation in introduced feral Rock Pigeons within the Eastern United States. Pigeons and humans have a long history of interactions and this history makes pigeons an ideal species to examine for phenotypic changes as a result of range introductions.

A Brief Natural History of the Rock Pigeon

The Rock pigeon (Columba livia) is an old-world pigeon used as a model organism for many processes (Levi, 1996), showing extensive morphological variation in the wild and in captivity. In the bird's native range (Southwest Asia, Northern Africa, and Western Europe), twelve subspecies are recognized, some of which have very limited ranges, such as the Dakhla and Kharga oases of Egypt (C. l dakhlae) and the Canary Islands of Spain (C. l. canariensis) (Gibbs et al., 2001). Changes in the physical traits of Rock Pigeons in the wild can be swift and conspicuous. For instance, the ancestors of the Canary Islands subspecies (C. l. canariensis) likely colonized these islands around 130,000 years ago when islands became habitable for large Columbiformes due to complex soils capable of sustaining grasses and rock structures such as small caves and ledges for nesting (Rando, 1999). The Canary Islands Rock Pigeon differs from their sister taxa (C. l. livia) in its size, coloration of the wing coverts, wing shape , and tail color (Gibbs et al., 2001). These examples of changes in phenotype observed in wild pigeons suggests that feral pigeons might change their phenotypes in response to variable conditions in their introduced ranges. Similar, introduction events of other Columbiformes, where a limited number of escapees have colonized and dominated a landmass, have occurred with the Eurasian Collared Dove (Streptopelia decaocto) in North America (Hengeveld, 1993) and the Zebra Dove (Geopelia striata) in Hawai'i (Richard and Fleischer, 1989).

A Brief Unnatural History of the Rock Pigeon

In captivity, selective breeding creates more dramatic variations to morphology. Pigeons were first kept by humans about 5,000 years ago as a food source, as pets, as messenger, and as religious icons (Gilbert et al., 2020). The first depictions of novel captive bred phenotypes were illustrations of common captive color forms including what are known by modern pigeon keepers as checkered and ash red (Facsimile Painting from the 'Green Room') and white (Scene in the Nile Marshes) birds from Ancient Egyptian tomb paintings, stretching back as far as 1353 BC. However, due to the long history of humans and pigeons, physical modification, especially in coloration, may have occurred earlier. A record of the most dramatic change to the Rock pigeon comes in the year 1238 when ancestral members of the tumbler pigeon breed were recorded by Europeans for the first time as "Syrian" pigeons. It can be noted, however, that these pigeons likely existed long before European documentation (Johnston, 1998). Tumbler pigeons are noted for their ability to perform "somersaults" in the air, often uncontrollably. This leads to either the unusual "flipping" of a flying bird close to the ground or the "cartwheeling" of a bird high in the air. While the reason for this unusual "tumbling" response is unknown, it is widely believed to be the result of either a heritable condition similar to Ménière's disease in humans or a neurological or behavioral alteration (Mowrer, 1940).

In modern pigeon keeping, new breeds can arise within a few years in the hands of a single dedicated breeder. One example of this phenomenon is the origin of the breed known as the "almond brown". This bird was bred exclusively by a keeper named W.F Hollander at Iowa State University from a "mixed stock" of birds and was used to study the sex-linkage of traits. The almond brown breed is known for its dark eyes as an adult, pink eyes as a juvenile, and pale brown plumage (Levi, 1996). Changes like these give further testament to how quickly (i.e. within a human lifespan) pigeon phenotypes can change.

In captivity, the number of extant domestic breeds is around 314. Some breeds have been selected for flying ability, others have been rendered flightless, while others can fly for 22 hours straight. Other breeds, bred for meat production, can weigh the same as Common ravens (Corvus corax) with weights up to 1400 grams, compared to the 238-302 gram weight of wild-type pigeons (Gibbs et al., 2001). Pigeons bred for showing and the pigeon fancy can have a variety of traits such as erect fanned tails, elongated and shortened bills, flight feathers on feet, hoodlike crests, and high-pitched calls (Levi, 1996). Among these modern breeds, birds have been produced with traits so foreign to their wild ancestors they often appear to be from a completely different family. It seems, given the right artificial selective pressures, that the plumages, vocalizations, behaviors, and anatomy of domestic Rock Pigeons have diversified to such a great degree that their multiplicity of forms, within this single species, surpasses even that of the entire family of the famously variable and bizarre birds-of-paradise (Paradisaeidae).

Rock Pigeons in North America

The history of pigeons in North America is vivid, rich and well recorded. The first Rock pigeons in North America were introduced in 1606 to Nova Scotia as free-flying birds by some of the first French colonists of the region. The English later separately introduced pigeons to the Virginia colony in 1621. While the exact reason for bringing these birds overseas is unknown, it may have been similar to the reasons pigeons were domesticated in the first place––for use as pets, messengers or food; or to produce guano. By 1773, Rock Pigeons could be found free-flying in some capacity (either as true feral birds or as captives kept in open dovecotes) from Nova Scotia south to what is now Florida and west to Illinois (Schorger, 1952).

Based on the folk-art depictions of pigeons and pigeon-like birds as well as dovecote designs from North America in the 1700s, the Rock Pigeons introduced to North America by the English and French were likely a form of the common utility and flying breeds kept in Europe at the time. These illustrations show popular pigeon breeds of the time without atypical plumage structures (Chimney Piece with Images of Adam and Eve). Pigeons were depicted in the air (Stone, Anstiss. “Memorial Brooch.”) and dovecotes rather than closed lofts were used for their housing (Kryder-Reid, 2020). These depictions show the early American pigeons as either pure white, implying the presence of at least some white domestic birds (Starr, Sarah. “Embroidered Sampler: Starr Coat of Arms.”) or gray with banded tails (Birth and Baptismal Certificate 1769). Although the exact breed or breeds that populated the Americas is unknown, depictions of commonly kept birds in France and England, showed that they may have been a mix of what is now called the Baghdad, dragoon, carrier, and, most likely an ancestral homer breed known as the “dovecote pigeon” (Levi, 1996). Other breeds such as barbs, pouters, (London Trade Card with Three Pigeons) and short-crested breeds, such as the archangel (Barlow, Diverse Pigeon Species) are also commonly depicted in the avian illustrations of Europe at the time (Buffon, “Little Dove, Powter And Dovehouse Pigeon”). Pigeons of these varieties were not recorded in the Americas until much later. Even if released in North America, the exaggerated physical traits of these breeds would make it unlikely they would survive and reproduce as other, less ornamented breeds might have. It can be reasonably assumed that the modern feral pigeons of North America are the descendants of these first European birds.

Rock pigeons, being introduced earlier than House Sparrows and having nearly the same range as introduced House Sparrows, could show similar levels of geographic variation in phenotypic traits. Feral Rock Pigeons in North America have been shown to be morphologically and genetically distinct from those in Europe. This is likely due to different source populations, rather than adaptive microevolution, as the "feral" European source population is likely a combination of domestic birds and true wild "Rock Pigeons" that the domestic birds may have bred with (Johnson, 1992). Different populations of feral Rock Pigeons from eastern North America have been shown to be genetically distinct (Carlen, 2020). This shows that at least at a genetic level, some geographic separation of populations has occurred, since the introduction of a possibly homogenous population. Despite the phenotypic variation expressed in both free-living wild pigeons and captive-bred individuals, no study so far has shown any distinct physical variation between populations of feral pigeons within North America.

The possibility for geographic variation in feral Rock Pigeons is evident in their ability to form subspecies in the wild and breed in captivity. This is further supported by the occurrence of phenotypic variation in species with similar patterns of introduction. There is already evidence of variation within Eurasian Rock Pigeon populations. The purpose of this study is to determine if similar variation is occurring within Eastern North America as well.

Methods

I captured feral pigeons from 19 different sites. Collection sites refer to places where at least one specimen used for this study originated. All applicable measurements (Figure 1) were taken on every captured or reviewed specimen. In both live and preserved specimens, some structures were not present (ie. cracked bills, missing primary feathers) values for measurements of these were simply left as 'null' for these specimens.

Table 1. The different measurements taken from the collected pigeons, and how they were done.

Measurement	Method	Description
Bill length	Electronic calipers (mm)	from the anteriormost point of nare opening to bill tip
Bill depth	Electronic calipers (mm)	from the ventral side of the lower mandible to the dorsal side of the upper mandible, taken at the deepest point
Head length	Electronic calipers (mm)	from the posterior-most point of the head to the tip of the bill
Tarsus length	Electronic calipers (mm)	from the joint of the tibiotarsus and tarsometatarsus to the hallux.
Grouse length	Electronic calipers (mm)	length of feathering from the joint of the tibiotarsus and tarsometatarsus to the phalanges
Wing chord	Wing ruler (mm)	from the carpal joint to the end of the primary feathers
Tail length	Wing ruler (mm)	from the beginning of under tail coverts to the tip of the longest present retrice
Overall body weight	Gram scale (g)	Highest recorded weight in the "weigh box"

Capture of Live Speciments

For individuals captured by researchers, four trapping methods were used: a commercial mechanical bow-net (Mikes Falconry, Gresham, Oregon, USA https://www.mikesfalconry.com), commercial Wire swinging-door cages (Tomahawk Livetrap, Hazelhurst, Wisconsin, USA https://www.livetrap.com/index.php?dispatch=pages.view&page_id=3), hand nets, and noose-carpets.

The bow net was staked down in a regular feeding and congregating area on the ground, then baited with the various foodstuffs (mostly commercial birdseed, but also bread crumbs and fried potato in some cases). A similar approach was taken with the wire swinging-door cages, but trails of bait were used instead of piles. Shade covers were applied to the traps when needed and water bowls were Provided. The noose carpet only captured one bird. In this case, the carpet was attached to a preferred perch, when the bird landed on the perch, its feet became tangled and could be easily removed. The hand net was only used in instances of heavy rain, when some birds were rendered flightless due to waterlogging of feathers. Any bird with poor flight abilities was captured on foot, measured, dried off, and released. On live birds, weight was measured by placing the captured bird in a 27x13x13.5 cm cardboard box with velcro on its opening flaps. The box was tared on a gram scale, the bird placed inside. Once live birds were captured, they were brought to a measurement site. This site was walking distance from the capture site, but obscured from the view of a bird at the capture site. Birds were transferred in a commercial dog carrier. The bottom of the carrier was covered in paper, which was changed for each group of pigeons captured. The carrier was also cleaned with vinegar every day after trapping was completed. After measurement, a V-shaped mark was cut into the bird's first pair of rectrices, so if recaptured, the bird could be identified and not re-measured. If a bird was captured missing the first pair of rectrices, it was released and not measured. All capture was approved by local agencies if applicable:

- Massachusetts Department of Conservation and Recreation no. R-149
- Maryland Department of Natural Resources permit no. 57400

Florida Fish and Wildlife Conservation Commission Permit no. EXOT-19-105

Deceased specimens

Frozen specimens were not weighed due to desiccation and added ice weight. If weight while living/recently deceased was noted on the label of a specimen, then it was recorded and treated as a measurement gathered by the researchers. Round skins, wet specimens, frozen specimens, and skeletons were utilized from the following institutions:

- *The Field Museum of Natural History (FMNH),*
- *The American Museum of Natural History (AMNH),*
- *The Academy of Natural Sciences in Philadelphia (ANSP),*
- *The National Museum of Natural History (Smithsonian) (NMNH),*
- *The North Carolina Museum of Natural Sciences (NCMNS),*
- *The Florida Museum of Natural History (FLMNH)*

Additional frozen specimens were utilized from Avian Haven, a wildlife rehabilitator in Freedom (near Augusta), Maine. No specimen older than 1970 or bearing yellow juvenile "fuzz" was used.

Statistical approach

With the exception of one site (Chicago, Illinois), I measured feral Rock Pigeon traits along a north-south transect from Augusta, Maine in the north to Key West, Florida in the south (Fig. 2). To test for differences in pigeon phenotypes across sampling sites, I regressed the phenotypic trait of interest on the latitude where the pigeon originated. Significant differences in traits across geography were revealed by trend lines with slopes that differed significantly from zero. I present regression plots for all traits examined and add lines to plots to illustrate statistically significant (or near significant) patterns in traits across sampling sites. To account for running a series of tests on multiple traits, many of which are related to one another, I corrected for the increased probability of detecting a significant relationship using false discovery rates, following Pike (2011).

Figure 2: Sites where measurements were taken, with locality names and number of specimens measured at the site.

Results

Overall, the bow-net was the most successful trap for capture. In total 199 live and preserved specimens were measured. Of physical traits measured, tail length, bill length, bill depth, and tarsus length showed no statistical differences between sampling sites (Table 2). By contrast, body mass, head length and wing chord were all significantly correlated with latitude, with larger traits (i.e. heavier body masses, longer head lengths and longer wing chords) observed in northern sites (Table 2). Grouse length tends to increase with latitude, however this result is close to, but not statistically significant (p = 0.056, Table 2).

Uncommon phenotypes. At the Key West collection site, I observed two rare phenotypes among feral birds: the “crested” (a short, curving crest behind the head) and “split eye” (black spots on the iris). These individuals were unbanded and, other than these mutations, appeared to be feral, making it unlikely that they are escaped, domestic individuals. I also observed a third possible mutation, known as “green eye”, however, its validity is questionable due to other factors such as age that can cause a similar appearance. Several free-living domestic birds were also found, but only one was observed behaving in a manner that would suggest possible interbreeding with the true feral population (an apparent pair bond).

Domestic escapes. Several escaped domestic, color-banded individuals were encountered and their breeds identified. The breeds and numbers of each breed encountered were: three racing homers, one Canadian tippler, one utility Strasser, and one Birmingham roller.

Table 2. Summary of regression analyses for all feral Rock Pigeon traits measured. Bold font indicates traits that differed significantly or near significantly at the 0.05 threshold.

Trait	#Individuals measured	Slope	SE	T-value	P-value	Adjusted p-value
Grouse length (mm)	162	0.108	0.056	1.929	0.056	0.074
Mass (g)	135	2.004	0.611	3.28	0.001	0.002
Tail length (mm)	171	0.13	0.135	0.963	0.337	0.317
Bill length (mm)	195	-0.009	0.011	-0.815	0.416	0.317
Tarsus length (mm)	195	-0.018	0.023	-0.804	0.422	0.317
Wing chord (mm)	181	0.365	0.098	3.736	0.0003	0.0008
Head length (mm)	196	0.137	0.025	5.584	<0.0001	<0.0001
Bill depth (mm)	192	0.0002	0.006	0.036	0.971	0.637

Discussion

The phenotypic traits that varied significantly between feral Rock Pigeon populations include body mass, wing chord, and head length. Grouse lengths trended higher in more northern populations, but was statistically insignificant. While these differences could be due to genetic drift alone, this is unlikely the case. The short time in which these differences must have emerged is not consistent with typical patterns of genetic drift, as well as the large population sizes present (Masel, 2016). It is possible, however, that genetic drift could have occurred in the early days of colonization, when feral populations were presumably smaller than they are today. Sexual selection is also a possible driver for these differences, but this possibility has not yet been investigated, and there is limited knowledge of the sexual selection methods of pigeons in the case of non-behavioral phenotypes. Below, I detail possible explanations for the geographic patterns in phenotypes observed across localities.

Traits that scaled positively with latitude

Body mass, head size and wind chord are all positively correlated with latitude (Figure 3). This pattern is consistent with Bergmann's Rule, which states that within a species of endotherms, individuals from populations in warmer climates will be smaller, while those in colder climates will be larger (Ashton, 2002). Bergmann's Rule holds that larger-bodied animals can retain heat more efficiently, while smaller animals can radiate heat more effectively. This pattern is manifested in about 72% of bird species (Meriri, 2003), and is most common in non-migratory species like Rock Pigeons (Meiri, 2003). True wild Rock Pigeon subspecies follow this pattern, while body weight is extremely variable in domestic breeds (Johnston, 1992).

Another trait showing positive variation was grouse length, the length of leg feathers found on the pigeons (Figure 4). The function of leg feathers in modern birds is varied and includes thermoregulation (Johnson, 1968), sexual display (Schuchmann, 1979), locomotion (Hohn, 1977), and other unknown functions. In Columbiformes, tarsi that are at least partially feathered are represented in green (Treron), mountain (Gymnophaps), topknot (Lopholaimus), New Zealand (Hemiphaga), imperial (Ducula), blue (Alectroenas), fruit (Ptilinopus), brown (Phapitreron), and olive pigeons (Columba). The function of these feathers (often found on tropical, tree-dwelling species) is unknown (Levi, 1996). In pigeons, it seems that thermoregulation is a possible selection pressure favoring leg feathering, as Rock Pigeons are known to lose significant heat through

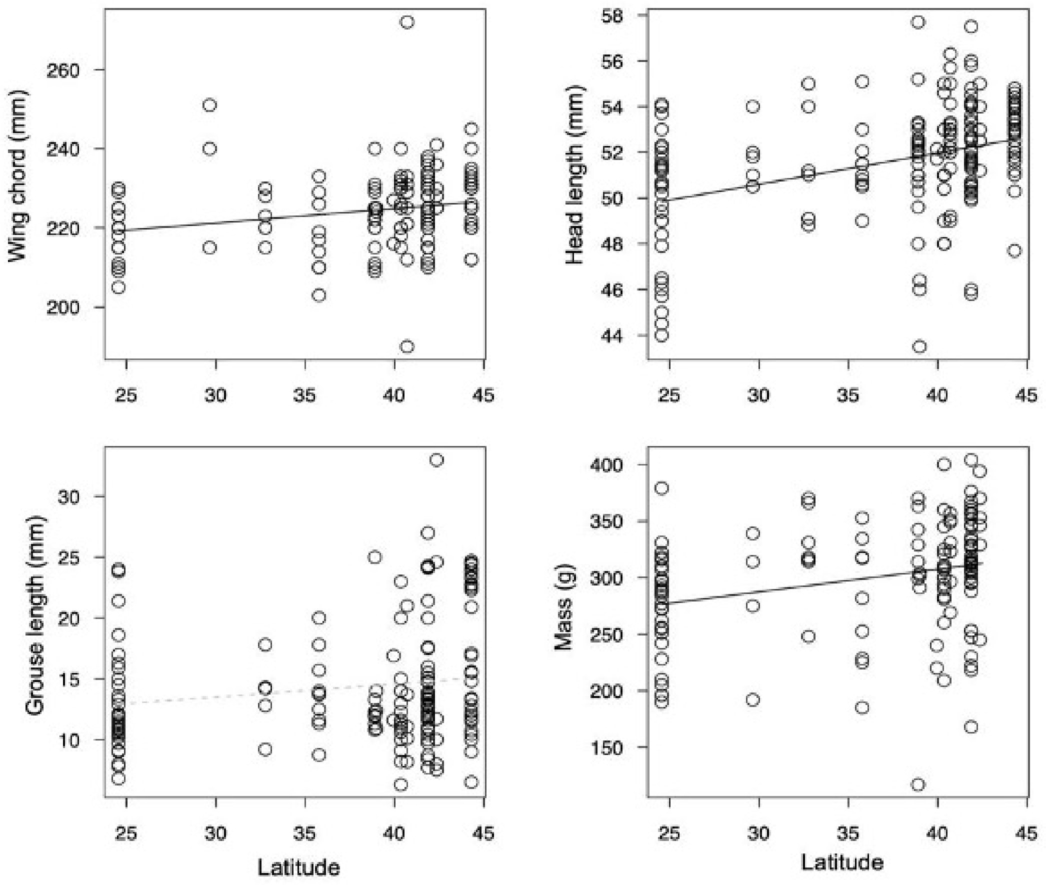

Figure 3. Regression plots of significant characters.

Figure 4. Variation in grouse length of feral pigeons

their legs (Martineau, 1988), suggesting that individuals in cold, northern climates might benefit by increased leg feathering.

Of the traits that showed no patterns with latitude (tail length, bill depth, bill length, and tarsus), some of these findings were surprising (Figure 5). For example, tarsus length and other linear traits often scale with body size and thus would have been expected to increase with heavier body mass at northern sites. Similarly, bill length might have been expected to increase in ways similar to head length. There are a number of explanations for why some traits but not others might vary with latitude. Traits that do not vary with latitude may not be important for coping with different environmental conditions, may be optimized for other functions (i.e., sexual display, which does not vary geographically), or may not have sufficient variation to respond to geographic variation in environmental conditions.

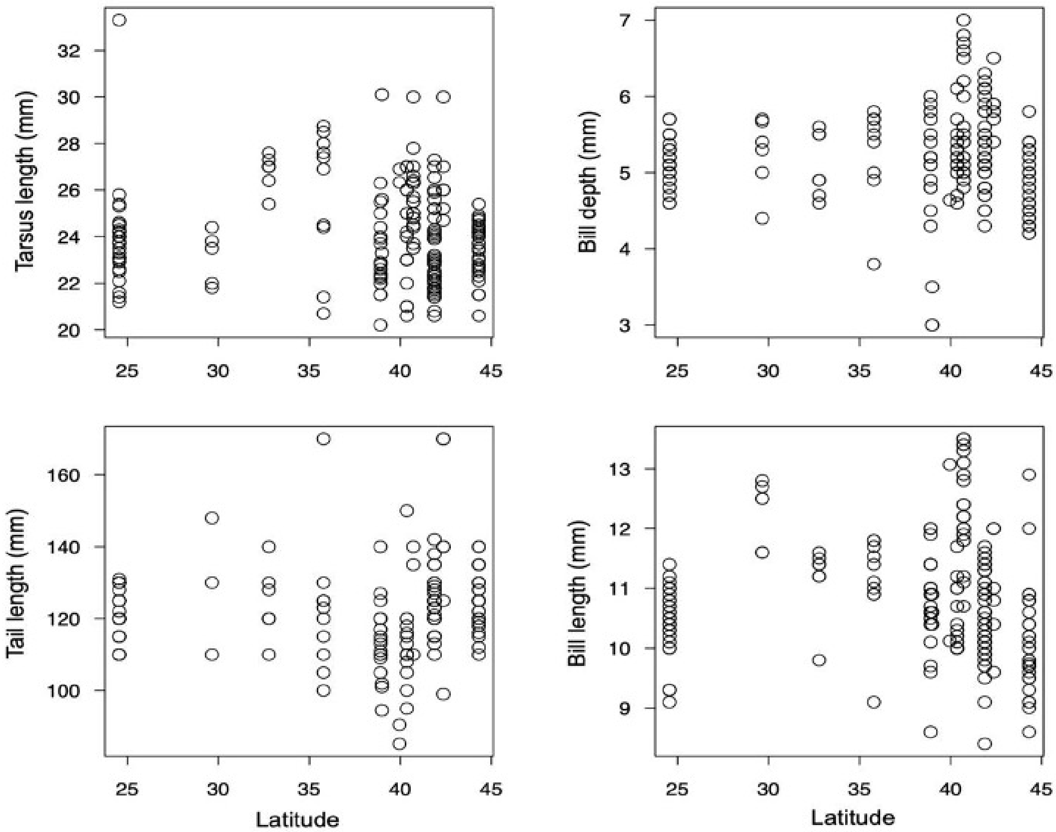

Figure 5. Regression plots of insignificant characters

Uncommon phenotypes

The two confirmed uncommon phenotypes "split eye" and "peak crest", were seen at the Key West trapping site (Figure 6). A third, unconfirmed phenotype "green eye" was also seen in Key West, but the validity of this trait as a mutation, rather than an unusually prominent but typical neotenic feature in a young pigeon, is uncertain. A heritable trait, "crest" is a common mutation seen in domestic pigeons caused by a point mutation of the EphB2 gene, where an arginine base is replaced by a cysteine base. This then causes feathers on the back of the head to grow in "backward" with tips pointing towards the bill rather than the tail producing a crest (Shapiro, 2013). Due to the sheer simplicity of the mutation needed to produce this phenotype, it is likely it could arise spontaneously within a population. Mutations of eye color are less well known. "Split eye" refers to the presence of patchy black in the iris, and is an uncommon but regular trait in many domestics. It has been postulated by pigeon keepers that split eye is a result of an improper migration of the typical colored pigment on the surface of the iris, exposing the dark lower layer. However, no studies have confirmed this, and nothing is known about the heritability of this trait (Muntaz, 2017). The "green eye" trait, defined as the presence of green in the iris, is rare among domestic birds. Only a handful of domestic breeds (such as the Syrian Tarbesh and Madrasi Highflier) have green eyes as a regular trait (Walker, 2011). In domestic racing pigeons, this mutation is very rare. In the United States, an otherwise undistinguished partially green-eyed bird was recorded as sold for 300 USD (CBS pigeon, 2020). Nearly nothing is known about the mechanisms of production or inheritance for this trait. The green-eyed individual captured for this study appeared to have this very rare trait; however, it was a hatch-year bird with its eye color not fully developed. While other hatch-year birds at the site lacked green eyes, the green seen on this bird might have been a juvenile trait that would fade with age.

Figure 6. Unusual phenotypes, left to right, crest, split eye and green eye.

Intrusion of domestic individuals

Of the domestic individuals found the Birmingham roller, identified by body shape and visible "rolling" aerial phenotype was the only domestic escape showing any potential breeding behaviors (such as allopreening and visiting potential nest sites). It is not known how long the Birmingham roller had been living feral or whether it has produced offspring. However, the bird appeared to be pair-bonded with a feral bird, suggesting the roller has been living among feral birds for at least three months (the minimum length of time for the formation of a pair-bond) (Wosegien, 1997). Another factor in determining the status of this bird was the condition of its toes. Part of one hallux was missing and one phalange was severely damaged, likely due to a condition called stringfoot, where a toe is tangled by hair or string and is then amputated due to a lack of circulation (Jiguet, 2019.) Birds in human care usually have objects tied around their feet removed, again pointing to this bird being outside of human care for some time. Other feral pigeons at this site also had apparent stringfoot injuries. Because no other pair-bonds were seen between feral and domestic birds, this does not appear to be a common phenomenon. Therefore, it is unlikely that the variation between populations of feral pigeons is heavily influenced by interbreeding with domestic pigeons.

Conclusion

Rock pigeons have been living in the US for about 400 years and, despite this relatively short time period, have a robust population and thrive throughout the continental US. This study revealed that introduced Rock Pigeons in the eastern United States show significant morphological variation between their populations. Pigeon traits change across latitude in ways consistent with Bergman's Rule, whereby populations in colder northern sites had traits indicative of larger individuals. The patterns in phenotypic variation I observed in feral pigeons are consistent with patterns observed in wild pigeons and in other species of birds. It is possible that the disparities in traits observed across pigeon populations will continue to widen, and further the Rock Pigeon's contemporary evolution.

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A Literature Review Applying the Social-Ecological Framework for Black Children with OSA

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Abstract

The purpose of this literature review is to compile an up-to-date overview of the academic literature on the parental detection of OSA in children and its subsequent diagnosis and treatment. It serves as an outlook into racial health disparities that exist in this area of research. The existing literature serves as evidence for this phenomenon, which is explored using the social-ecological model as a framework for inquiry. A literature search was performed using The National Library of Medicine and Google Scholar databases between July and August 2020 and August 2022 to assess the various factors affecting parental detection of obstructive sleep apnea in their children. Different combinations of keywords including but not limited to "health disparities," "racial disparities," "racial differences," "pediatric OSA," "pediatric obstructive sleep apnea," "OSA treatment," "OSA diagnosis," "OSA detection" and "parental experiences" were used to find relevant studies and reviews. There were approximately 30,000 hits from these terms. The inclusion criteria were peer-reviewed literature published within the last 10 years, except if the paper included the etiology of the disease. Children's access to care can act as the inclusion criteria for examining studies. Children between the age 2 and 12 years old, as well as adolescents below age 18, were considered. The main extraction of data from the primary studies were risk factors of pediatric OSA, the foundation of the socio-ecological framework, the detection process of OSA and racial differences among pediatric populations. OSA knowledge is important for detection by parents, family members, and teachers who are constantly around children. They are the main interpersonal supports that detect OSA and prevent delay in detection. The community support roles are health care providers and specialists that can monitor the progression of OSA and ensure adequate treatment. Additionally, the access of children to care can act as a barrier of OSA treatment. Black children experience pediatrics OAA severity more than their white counterparts based on the factors outlined in the social- ecological framework.

Introduction

Approximately 3% of children are affected by obstructive sleep apnea (OSA) (Chang & Chae, 2010). Nevertheless, racial and ethnic minority children are affected up to six times more than white children. African-Americans, Hispanic-Americans and Native-Americans have higher prevalence of OSA than Asian-Americans and Caucasian Americans. African-Americans are 4-6 times more likely to have OSA than white children. One study shows craniofacial structure, genetics and obesity explain prevalence differences in OSA prevalence between races, contributing to African-American OSA disparities, which leads to the worst reported treatment outcomes (Dudley & Patel, 2016). The degree of OSA severity is higher in Hispanic-Americans although data is limited. Native-Americans have moderate to severe OSA approximately 1.7 times greater than whites. Meanwhile, Asian-Americans report a lower occurrence of pediatric OSA (~18% to ~36%) than whites (Dudley & Patel, 2016). The etiology of pediatric OSA has been attributed to inflammatory factors and biomarkers, craniofacial abnormalities, genetics, allergic rhinitis, adenoid or tonsil hypertrophy and obesity. There are two types of pediatric OSA: type 1 is distinguished by marked lymphadenoid hypertrophy without obesity and type 2 is associated with airway lympha-denoid hyperplasia with obesity (Capdevila et al., 2008). Adults and children have distinct levels of physiology which includes, but is not limited to, respiratory function, sleep physiology, thoracic mechanics and upper airway development that may lead to various manifestations of OSA (Alsubie & BaHammam, 2017). It is recommended that children are screened for snoring, via a polysomnography (PSG) study or an alternative diagnostic test should be performed such as an adenotonsillectomy (T&A). The first line of treatment for adenotonsillar hypertrophy (ATH) patients is the leading cause for OSA. Otherwise, continuous positive airway pressure (CPAP) is used as the standard treatment. High-risk patients must be monitored post-operatively and weight loss should be considered with other therapy in obese children (Marcus et al., 2012). These disparities will magnify unfavorable health outcomes if left untreated. Obstructive sleep apnea is defined by repeated cessation of breaths during sleep. In 1976, the pediatric condition was distinguished from the disorder found in adults (Huang & Guilleminault). Adult and pediatric OSA differentiate in causes, risk factors, polysomnography (PSG), treatment, and co-morbidities. Therefore, they must be considered separately to prevent deleterious consequences. Consequences of untreated pediatric OSA may include sleep disruption, cardiovascular problems, endocrine issues, snoring, repetitive apneic episodes, daytime sleepiness, metabolic systems difficulties, neurobehavioral dysfunction, and hypoxemia (Spilsbury et al., 2006) (Capdevila, Kheirandish-Gozal, Dayyat, & Gozal, 2008) (Strocker & Shapiro, 2007). These comorbidities may contribute to poor academic performance. The under-detection of childhood OSA leads to under-diagnosis, developmental problems, and amplified health disparities.

Pathways and Frameworks for Pediatric OSA Health Disparities Research

The socio-ecological framework for prevention contemplates the interplay between individual, relationship, community, and societal factors that cause health risk for populations (Krug E, 2002). These factors overlap and influence each other continuously. Health disparities are interwoven in social, environmental, behavioral, and biological influences (Roux, 2012). Roux outlines five models for exploring research methods in health disparities which include the genetic model, the fundamental cause model, the pathways model, and the interaction model, which considers the interconnected mechanisms that impact these disparities . His approaches highlight the importance of selecting a conceptual framework that leads to the necessary research questions, hypotheses and systems that tackle the OSA disparities problem. The main takeaway is that the factors previously outlined are comparable with the social intervention frameworks described in the social-ecological model of health. The social-ecological model is a six-level framework for prevention that integrates the individual, relationship,organization, community, and societal factors that lead to public health influence (Dahlberg LL, 2002). Micheals (2022) stated that these factors are broad contributors of disparities that may collectively provide insight into the status of OSA experiences among Black children. In recent research, the six levels of the social-ecological framework have been applied to sleep health in families of young children to prevent poor sleep quality. A recent review summarizes the ethnic, racial, and socioeconomic disparities related to sleep-disordered breathing (SDB) (Williamson et al., 2022). The following were the socio-ecological levels analogous to the framework explored in this review: neighborhood and broader socio-cultural factors (societal and policy), healthcare setting factors (organizational), school setting factors (community), family factors (interpersonal) and individual factors like our model. Another study explored the socio-ecological framework to understand adherence to the positive airway pressure (PAP). The socio-ecological model was helpful to understand and identify predictors of pediatric continuous positive airway pressure (CPAP) adherence and determine possible treatment for OSA (Xanthopoulos et al., 2021). The socio-ecological model is a steady model for exploring predictors, barriers, and treatment of obstructive sleep apnea in children.

The socio-ecological model organizes factors causing disparities of OSA symptoms, diagnoses, and treatment delivery. Societal and lifestyle factors have been studied in previous research. For instance, neighborhood disadvantage is a community (and environment) factor that explains racial differences that affect waking after sleep onset (WASO) and sleep efficiency (Fuller-Rowell et al., 2016). A study by Spilsbury et al. (2006) compounded primary caregiver education, household characteristics, and race amongst other components of neighborhood disadvantage, that found a significant relationship within the African-American subgroup (Spilsbury et al., 2006). Single-parent households, public insurance, and proximity to care centers were determined by sociodemographic and socioeconomic status (Xie et al., 2018). The proximity of participants to the medical center in the study determined the prevalence of OSA. Thus, the nearer families lived,the less there were occurrences of OSA in their children, illustrating the importance of access to care at the organizational level of the socio-ecological framework The theoretical framework follows the Centers for Disease Control and Prevention Socio-Ecological Model (SEM): A Framework for Prevention (CDC, 2020). It is posited that the factors that contribute to pediatric OSA disparities can be represented at each level of the SEM framework, and that intervening upon multiple levels of this model is needed for effective OSA prevention and treatment strategies to narrow the pediatric OSA health disparities gap.

Methodology

A literature search was performed using National Library of Medicine (PubMed) and Google Scholar between July and August 2020 and updated in July 2022 to assess the various factors affecting parental detection of obstructive sleep apnea in their children. The following keywords and search terms were used during the search: “Obstructive Sleep Apnea in Children”, “Parental Experiences with Obstructive Sleep Apnea”, “Racial Differences in Obstructive Sleep Apnea”, “OSA Detection in children”, “OSA in children and parental experiences”, “Disparities in obstructive sleep apnea”, “Parental experiences with OSA” and “Racial disparities and children with OSA”. Different combinations of these search terms generated 30,000 articles. The articles that were included were published in the last 10 years and if not, they described important etiology of OSA and improved racial/ethnic relevance with OSA. The inclusion criteria for examining studies were studies that included children between the age 2 and 12 as well as adolescents below age 18 to be considered. The main extraction of data from the primary studies were risk factors of pediatric OSA, the foundation of the socio-ecological framework, the detection process of OSA and racial differences among pediatric populations. The interventions and claims were formed based on the main findings of primary articles in Table 1 and key socio-ecological factors outlined in Table 2.

Results

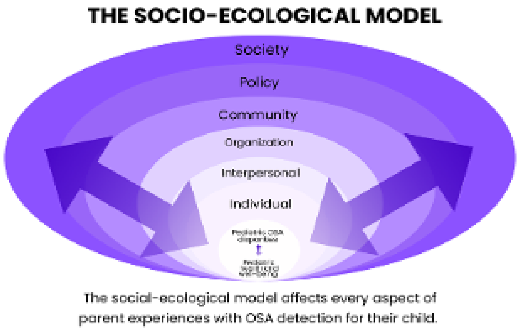

Figure 1. The Social-Ecological Framework showing overlapping levels contributing pediatric OSA health disparities. This diagram shows the social ecological model as a framework that shows the factors affecting OSA detection and treatment in Black children. Adapted from Krug E et al., World Report on Violence and Health. Geneva, Switzerland: World Health Organization; 2002:1-21

Levels of the Social Ecological Framework: Intervention

Individual Level

The individual is the primary pillar of the framework includes the biological and personal history that affects OSA detection as shown in Table 2. To improve the diagnosis of OSA, the knowledge and awareness of parents about their child's sleep must be changed. There must be individual awareness, knowledge, or prior experience about a biological predisposition. Delayed detection may be due to the parents' lack of awareness. Snoring may be normalized and not necessarily a point of concern which may delay the detection process (Honaker et al. 2022).

Interpersonal Level

The interpersonal level includes parent experiences,social connections and healthcare provider support which is outlined in Table 2. To strengthen the child's support system, the patient-provider relationship must be built with trust. The communication threshold (low to high) determines the likelihood of OSA detection at an early age. Implicit bias often acts as a barrier for patient experience, care, and treatment especially among Black patients. A study on this topic found that implicit bias was significantly related to patient-provider treatment decisions, interactions and patient health outcomes (Hall et al., 2015). Moreover, research shows that Black patients experience discrimination and provider bias that affects treatment adherence (Williams et al., 2022). Also, parents can learn from their family members which may further increase their own knowledge and awareness.

Organizational Level

At the organizational level the child's teachers, social worker and or healthcare provider (such as a dentist) must have the ability to identify and detect symptoms of OSA in the child. Research shows that teacher-reported child sleepiness was associated with lower adaptive behaviors, more problems in the classroom and lower academic achievement, especially in Black children (Ursache et al., 2021). Thus, their observation of children's behavior is crucial for OSA diagnosis and treatment. Moreover, Honaker et al. (2022) stated that teachers identify sleep disorders in children such as daytime sleepiness which may be an indicator of sleep problems that affect classroom achievement.

Community Level

The support of parents, health care providers, specialists and teachers are pertinent for OSA detection (Honaker et al., 2022). Table 2 shows the breakdown of parent experiences, social connections and healthcare provider support. To support the parents and their child, OSA education during pregnancy, post-partum and before enrollment in school is imperative. If the parents realize there are symptoms related to poor sleep quality, they may be better equipped to advocate for their child.

Policy Level

Those that live closer to health centers find that they have lower rates of OSA in their children (Spilsbury et al., 2006). Thus, the enactment of policy that requires sleep apnea screening at a minimum age for free or at a reduced cost for children under their parent's insurance can help mitigate the neighborhood disadvantage. Moreover, clinics in metropolitan cities may be

Figure 2: The social ecological model showing overlapping levels contributing pediatric OSA health disparities in expanded form with framework subcategories.

helpful for the attraction of parents and their children from different zip codes to seek screening.

Society Level

The societal level includes broad factors such as social and cultural norms, economic standing, technology, media, and resources available. These factors require systemic changes by organizations such as the World Health Organization, fundraising established by non-profit organizations and governments, donations from private corporations and online or in-person education programs for communities.

Table 1: List of key studies and reviews outlining the social-ecological factors (society as the main contributor) associated with pediatric sleep apnea and their main findings

Author, Year	Sample Size	Framework Category	Main Findings and Outcomes
Xie et al, 2018	105 patients with Refractory Obstructive Sleep Apnea and 52 patients with Obstructive Sleep Apnea	Community Organizations	Despite higher rates of single parent households and public insurance, those patients that lived closest to our care center had lower rates of OSA, suggesting how access to care can affect outcomes
Kendzerska et al., 2016	659 participants	Society	A potential 27% improvement in CPAP acceptance associated with higher neighborhood income not ignorable
Marcus et al., 2012	N/A	Society Community	All children/adolescents who snore should be screened for OSA. PSG should be performed in children/adolescents with snoring and symptoms/signs of OSA; if PSG is not available, then alternative diagnostic tests/specialist referral should be considered. Objective testing should be performed in patients who are high risk or have persistent symptoms/signs of OSA after therapy. CPAP is recommended as treatment if adenotonsillectomy (T&A) is not performed or if OSA persists postoperatively . Weight loss is recom-mended for overweight/obese patients
Strocker et al., 2007	584 patients	Society,Community, Individual, Interpersonal	Sleep disordered breathing and OSA can affect a child's physical and mental health. Parent and child quality of life may also be affected. Poor screening of children for the more subtle signs of disordered breathing such as

			snoring, daytime sleepiness, frequent awakenings during sleep, may result in reduced evaluation and treatment to prevent poor academic performance or behavioral problems. If pediatricians are more aware of the spectrum of severity of sleep disordered breathing and its impact on quality of life, there are more likely to screen children and, when necessary, refer to sleep specialists and/or otolaryngologists for diagnosis and treatment.
Honaker et al., 2022	30 parents	Society, Community, Organizational, Interpersonal	Parental experiences with OSA were attributed to the health care system, parent willingness to report, knowledge of night-time and daytime symptoms and overall OSA knowledge.
Goldstein et al., 2011	364 children	Society, Individual	Black race and prematurity were associated with snoring.
Stephanski et al., 2008	198 children and adolescents	Individual	African-American children with SDB has greater oxygen desaturation with obstructive dealings as compared to Caucasian and Latino children. African-American children experienced increased risk for hypoxemia and cardiovascular consequences of SDB.
Boss et al., 2011	33 children	Society	Black children (12/33) and children with poor socioeconomic status were the most studied. The children with higher prevalence and increased risk for SDB were in racial or ethnic and socioeconomic minorities. White children with private insurance were most likely to undergo an adenotonsillectomy (T&A).

Table 2: The Categories and subcategories within the social-ecological framework

Society	-Public health initiatives -Trustworthy media influence (or lack thereof)	-Overall quality of life -Poverty -Societal conflict -Racism, oppression, xenophobia, marginalization, implicit bias, and discrimination -Stigma and bias -Socio-economic status	-Environmental forces	-Technology and innovation
Policy	-Research and reporting gaps	-Federal, statutory, and local regulations	-Government resource allocation in research and development	
Community	-Research and reporting gaps	-Federal, statutory, and local regulations	Government resource allocation	
Organization	-Parent or guardian education level	-Social Environment	-Healthcare System -Accessibility to Health Systems & Health services -Health coverage Provider linguistic and cultural competency -Quality of care and PCP accountability -Two-sided Health Communication between PCP and Patient (Patient Provider relationship)	-Zip code/ neighborhood
Interpersonal	-Parent Experiences -Psychosocial status -Stress -Work-family Conflict -Racism -Sexism -Self-care -Mental health/disorders	-Social Connections -Parent-child relationship -School nurse -Counsellor -Teachers -Coaches -Peers	-Healthcare provider support -Telehealth -In-person physician support -In-person nursing support	
Individual	-Personal characteristics -Identity	-Biology and Genetic Factors	-Stress Response	-Race -Sex -Body Mass Index (BMI) - Genetic Endowment

Analysis

The socio-ecological framework is the chosen model for understanding the health disparities in parental experiences with their child's OSA in this paper. There are distinguished features such as the socio-ecological model to recommend possible intervention approaches based on the disparities that exist among ethnic minorities. This review is the first to address the disproportionate detection of pediatric OSA in marginalized groups by suggesting pathways and frameworks to curtail the deficiency in research of Black children. The claims are that health disparities are exacerbated because of the factors contributed by the socio-ecological model. Several studies' findings elucidated the current factors of pediatric OSA and left space for future research studies to tackle the field of knowledge.

Heterogeneity among races: Neighborhood disadvantages and genetic predispositions of African Americans

The purpose of the review is to highlight that the current literature displays heterogeneity among races within the cases of pediatric OSA. There is a research gap that exists in pediatric OSA and even more so related to racial and ethnic differences. According to Goldstein (2011), "neighborhood of severe socioeconomic disadvantage was significantly associated with OSA after adjusting for effects of prematurity, obesity, and African American race." Based on Table 2, Goldstein made connections between individual, community and societal factors that determine OSA outcomes. Interestingly, Wang et al. (2017) focused on using neighborhood variables to explain race and OSA associations and found that neighborhood socioeconomic variables provided a better explanation for racial disparities in pediatric OSA. Thus, society and community are socio-ecological key factors that increase risk of pediatric OSA. Goldstein (2011) found that there is a high prevalence of families of patients with OSA, whom likely have multiple genetic factors that cause the OSA phenotype phenotype. This refers to the genetic importance of the individual and their outcomes with pediatric OSA.

Roux's Models vs. The Socio-Ecological Framework: Pediatric OSA Study

The current literature highlighting genetic endowment and neighborhood disadvantage nudges a focus on the interaction model (role of gene-by-environment interactions) as explained by Roux, a relevant conceptual framework for future study. Scientists have found social factors that appeal to the interaction of environment and genes, which faces challenges such as broad causal models and the complexity of phenotypic acquisition (Diez Roux, 2012). The fundamental cause model relates the social and economic as primary causes of health disparities in pediatric OSA, which is highlighted by Goldstein. The articles mentioned previously show that there is an opportunity to focus on environment and genetics, any other subcategory outlined in Table 2 can be used as variables for health disparities research to develop the current field of pediatric OSA knowledge. As such, the socio-ecological model creates a comprehensive scope for examination since it recognizes that individuals are affected by a complicated range of social factors that are embedded in environmental interactions. Roux's models offer specialization for investigators who wish to dive deeper in the subcategories displayed in Figure 2. Thus, the socio-ecological model is the preferred framework for explaining in-depth factors that affect the health and well-being of children with OSA Pediatric OSA outcomes are heavily dependent on the interplay of society, policies, community, interpersonal relationships, and the individual patient (Honaker et al., 2022). The overarching societal level includes socio-economic and socio-demographic influences on the reality of children with sleep disorders. Research outlined in this review clearly shows that the type of insurance (public vs. private) determines the delay in treatment and controls the outcome of pediatric OSA. At the same time, community plays a crucial role in the health outcomes of pediatric OSA since children experience many forms of socialization that monitor their well-being. These may include the teacher, school nurse, pediatrician, dentist, and teacher which may increase the likelihood of detecting OSA. These individuals all serve as checkpoints for the child; thus, their knowledge of signs and symptoms is significantly important. Additionally, the family is the core of a child's support system as it does take a village to raise a child. The roles of parents are to monitor daytime and nighttime symptoms, advocate for their children, and ensure follow-up with their appointments. All these factors are controlled by the child's racial and ethnic identity which directly affect their health trajectory.

Recommendations and Implications

The research topic has been growing over the years, but current and continued efforts are underwhelming. As such, sleep scientists, academics and physicians should daycares, clinics and schools are he research of pediatric OSA is underwhelming, This calls for adjustments of educational programs in medical schools among other post-graduate health curricula to prevent the under-diagnosis of pediatric OSA. Also, there needs to be minority specific interventions and public health campaigns targeting these high-risk families to bridge the gap in knowledge and cultural behaviors. Future research can address gaps in research considering the effect of lack of physician knowledge on existing racial disparities of pediatric OSA. Racial disparities configure different problems for individual groups of OSA patients and their parents. Risk factors such as healthcare system, physical environment, economic stability, and community create unique contributions to OSA based on heterogeneity. Future research is needed to better understand the heterogeneity that exists within races, validation of screening instruments in non-white groups, a better understanding of potential differences in susceptibility to cardiovascular consequences of OSA, and the development of strategies to reduce the barriers to diagnosis and optimal treatment in minorities. Moreover, disadvantaged neighborhoods must focus on organizational and community health education and promotion. Thereafter, adequate interventions can be outlined based on unequal burden of OSA among races.

Conclusion

In sum, there are different experiences for children with obstructive sleep apnea. These differences are based on the individual awareness of parents due to lack of experience and knowledge. The family and patient-provider relationships can prevent delayed detection. Implicit bias can create distrust with parents that may reduce appointment attendance, follow-up, and overall treatment. The healthcare providers control the outcome of pediatric OSA by effectively treating children as required. The access to healthcare acts as an upstream factor that interferes with the first line of treatment which is a PSG. Overall, health disparities clearly exist in pediatric OSA, and the levels of the socio-ecological model can help elucidate these issues, especially among Black children.

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Abbreviations

OSA	Obstructive Sleep Apnea
CPAP	Continuous Positive Airway Pressure
PAP	Positive Airway Pressure
SDB	Sleep Disordered Breathing
ATH	Adenotonsillar Hypertrophy
T&A	Adenotonsillectomy
SEM	Socio-Ecological Model

Qué Bonita: A Remarkable Story of Family, Structure, and Love

By Juliana daRoza
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Abstract

Traditional bridal wear is often described as white dresses made for women to wear on the day they marry a man to become a wife. This description does not include the many different social identities and cultural variations that people inhabit. Today brides are not just looking for dresses or even white garments: today, people are looking for a special outfit to marry the person they love, whether that be a man or woman: they are looking for an outfit that will make them feel special and beautiful.

The collection is inspired by my grandmother, her love for her culture of Mexico, and her beliefs in the Catholic Church: creating my fascination with the insertion of the Catholic Church and Mexican culture. Plagued with colonization and destruction, a beautiful culture filled with vibrancy and joy was created, the Mexican Catholic Church. My story begins with my grandmother falling in love in Mexico, wearing none other than a white dress that changed her life. Once married, my grandparents moved to a farm in Northern California, where my grandfather planted a garden so vast it became a child's fantasy land and my grandmother's joy. There she raised her family and beloved canary birds, instilling the beliefs of the Catholic religion and stories of the Mexican culture.

Using practiced disruptive design techniques, I aim to transform the bridal industry into a space that's inclusive and empowering to both people and the environment, starting with the way we view ourselves and society's definition of beauty by drawing inspiration from culture. The collection was developed around the design philosophy of creating with the allure of drawing glamour and awe.

Through my inspiration and research from Mexico, the Catholic Church, my family heritage, and spectacle, this collection questions the importance of a dress and how to make people feel special. It will allow them to love themselves as they start a new chapter in their lives. As an esteemed bridal designer, Vera Wang, once said, "It's not just another dress, It's the dress you'll remember forever" (Wang, n.d.).

Proposal Summary

Traditional bridal wear is often described as white dresses made for women to wear on the day they marry a man to become a wife. This description does not include the many different social identities and cultural variations that people inhabit. Today brides are not just looking for dresses or even white garments: today, people are looking for a special outfit to marry the person they love, whether that be a man or woman: they are looking for an outfit that will make them feel special and beautiful.

Through the use of practiced disruptive design techniques, I aim to transform the bridal industry into a space that's inclusive and empowering to both people and the environment, starting with the way we view ourselves and society's definition of beauty by drawing inspiration from culture.

Through my inspiration from Mexico, the Catholic Church, their heritage, and spectacle, this collection will make people feel special, important, and confident. It will allow them to love themselves as they start a new chapter in their lives. As an esteemed bridal designer, Vera Wang once said, “It’s not just another dress, It’s the dress you’ll remember forever” (Wang, n.d.).

Design Philosophy

I aim to design with the allure of glamor and awe. The collection is inspired by my life experience at the intersection of the Catholic Church and Mexican culture.

My ambition for the collection is to demonstrate that bridal design is an art form that speaks to the fantasy of fashion at the highest level of luxury and haute couture. This collection is very personal to me: I want to honor both my cultural background and the broader culture of Mexico by creating a collection that is not only a work of art but also creates a space of Latina representation in fashion that as a young girl I wished existed for me.

Objectives

Upon completion of the thesis project, I will have gained an understanding of:

- The importance of research
- My family history
- My design development process
- The process of trial and error within the design process
- How to write a thesis design report
- How to produce a collection of eight professionally made looks using bridal and haute couture techniques
- How to appreciate a culture without appropriating it
- How to tell a story through design about the beauty of family and culture

The Muses

Muse One: The Catholic Church

Romans 13:8: *Owe no one anything, except to love each other, for the one who loves another has fulfilled the law.*

Love, peace, compassion, and reconciliation are a few of the values institutionalized by the Catholic Church. Infused with beliefs from the bible and tradition, the Catholic Church is one of the largest Christian denominations in the world and has an intense effect on the Western world-spanning ruling governments to architecture.

The Catholic religion is strictly hierarchically, structured with the Pope in Rome presiding over cardinals, who in turn preside over archbishops. Archbishops lead archdioceses which are split into parishes led by priests who interact directly with worshipers. The religion follows strict tradition-with only men allowed to enter the priesthood and women serving as nuns. The congregation of the Church has a goal of following and participating in the seven sacraments throughout their lives, one of which is matrimony.

During the Middle Ages, the papacy began to gain authority over the church. Missionaries worked to expand the faith beyond geographic boundaries. The largest, most dramatic, and most violent mission was the Crusades (Tyerman, 2005). Christian learning began to be incorporated within schools, with cathedral schools replacing monasteries which became universities (McKenzie, n.d.). By the 10th century, the institution of Catholicism had been planted, and the emergence of theology began (McKenzie, n.d.). Themes included the idea of God, humanity, the world, salvation, the divine, worship, and the study of the last times. Marked by decay and corruption, the 10th century marked a time of decadence for the Catholic Church, which became one of the wealthiest entities of the Middle Ages. By the 11th century, around the year 1000, many of the traditions still followed today had been established, including the sacraments. Life today for many Catholics is marked through the timeline of the sacraments (Evason, 2018). Although the church was built on what was believed to be good values, those opposing them were viewed as heretics and faced severe punishment, including death.

In the early 16th century, Mexico was conquered by the conquistadors, soldier-explorers of Spain, and allied countries led by Hernán Cortés (Riding, 2016). Alongside illness and death, the conquistadors brought the traditions of the Catholic Church (Evason, 2018) to Mexico. During the fall of the Aztec capital, Tenochtitlan, modern-day Mexico City, the now-enslaved Aztec people were forced to build churches for the Spanish. The first cathedral was built on top of El Templo Mayor, a temple in Tenochtitlan dedicated to Tlaloc, the Aztec god of rain, and Huitzilopochtli, the Aztec Sun and War God and God of Tenochtitlan (Kilroy & Zucker, 2017). This was one of the biggest sites for worship in the Aztec culture (Cohen-Aponte, 2021).

Figure 1. Front view of Mexico City Cathedral; Part of El Templo Mayor Ruins with from the back of the Cathedral

Due to the many similarities between the Catholic saints and the Mesoamerican gods, the conversion of people came fairly easy, especially because that the place of worship remained the same as seen in figure one. There are even folklore tales of underground tunnels connecting the Cathedral with one of the pyramids of the temple. (Cohen-Aponte, 2021)This mix of religions was the start of the Mexican Catholic church. Although architects for many of these buildings were Spanish, the craftsmen were Indian, creating a new mestizo style combining architectural styles (Cohen-Aponte, 2021). These awe-inspiring spaces were the start of the inspiration for my collection.

Muse Two: My Mexican Heritage

Figure 2. My maternal grandmother in her beloved garden

My grandparents immigrated from the state of Jalisco in Mexico in 1960. They moved to Vacaville, California, a small farming town now best known as a pit stop on the way to the world-class ski destination of Lake Tahoe. They raised a family of ten on a ranch on Rogers Lane, a street that became formative in many of the lives of their children. Both of my grandparents worked in the apricot and peach orchards in the Northern California area near Fairfield. In addition to strictly following the beliefs of the Catholic Church, my grandmother was extremely superstitious. She infused fantasy to me at a young age through both the stories of the church and Mexican fairy tales. I was told never to get my palm read because God's plan was the only plan. To say the sign of the cross whenever passing a cemetery is a way to pray for those lives. To care for the flowers like one would care for people. The idea and sanctity of life heavily influenced culture and religion in much of Mexican culture and certainly in my family.

Upon moving from the ranch to their first home, my grandfather built my grandmother a garden (figure 2); it was a child's fantasy land of cactus, pomegranates, apples, oranges, kumquats, figs, and peaches. The garden wrapped around the small one-story family home and my grandmother was always found somewhere hidden among the flowers and the fruits with her second children—her canaries. The birdcage sat in the center of the garden, next to two chairs under the apple tree and a large cross. As a little girl, I'd sit there with my grandmother in between playing hide and seek, scared to touch the cactus plant around the corner. She was the head of a strong matriarchy, followed by my mom, aunts, and older cousins, that shaped my family and the culture of my childhood.

Although my Spanish was never strong, I felt like we had our own language. I always understood what she said, "Mi Bonita."

Although we never fully celebrated the Day of Dead or Dia de Los Muertos, we followed many of the beliefs attached to the holiday. The holiday was a blend of Mesoamerican, European, and Spanish cultures (Editors, 2018). The tradition spawned from 'All Souls Day' a pagan celebration that the Roman Catholic Church adopted in Mexico as a way to blend cultures. Medieval Spain celebrated all Souls Day, bringing wine and pan de animas to the graves of loved ones in addition to flowers and candles (Editors, 2018). Conquistadores carried these traditions with them, aligning them with similar beliefs of ancient Mexican cultures. There are debates as to whether the holiday was endorsed by the Church to aid in the blending of culture or was celebrated by the Mexican people as a way to regain their cultural roots, separating away from the church. The Aztec and Nahua people of ancient Mexico believed in a cyclical view of the universe and saw death as an integral, ever-present part of life. My grandmother had a similar belief of life and death, although the holiday did not become popular in Western American Culture until recently. Children's films such as Disney's Coco and 20th Century Films The Book of Life explore the holiday and its importance in Mexican culture.

Muse Three: My Family Heritage

Figure 3. My baptism, the first Holy Sacrament of the Catholic Church (From left to right: dad, maternal grandmother, my paternal uncle and godfather, my maternal aunt and godmother, mom, me, paternal great uncle and priest, maternal grandfather, and paternal grandmother

As a predominantly Mexican-Catholic family, we attended mass every Sunday with my family and spent hours gazing into the beauty of the stained glass windows and never-ending columns. My knees would hurt from kneeling while viewing at the elaborate altars filled with candles and flowers.

We said grace at dinner. Attended events at our Church. Celebrated every religious holiday. The Catholic religion became a fundamental pillar of my childhood, bringing together both sides of my family as seen in figure three.

Drawing upon this background, my collection will highlight an intersection of culture, beauty, and representation. I plan to incorporate different aspects of hand and design work inspired by my Mexican Catholic heritage into my collection.

The Power of a Dress

The importance of a dress or piece of clothing can change one's perspective, it can encourage importance and confidence. It can become a part of one's family history. Designers hold power to break down barriers by creating a lens through which others can see the world. As someone who strives to make others feel seen and special, I intend to create a lens to improve humanity by disrupting design within the bridal industry. Through this change, we as designers have the power to make people of all identities feel important and confident in themselves, similar to the way my grandmother felt when putting on her white dress.

After joining the Mexican Military Services with his brother to support their family, my grandfather traveled throughout Mexico. In 1953, while still in the military, he traveled to Sayula, Jalisco, a larger town in the area hosting a festival.

My grandmother, after enduring a dark childhood with the loss of her mother and an abusive father, spent most of her time with her maternal grandmother. My great-great-grandmother spent much of her time sewing and helping prepare for their town's festival in Sayula. For the festival, she made my grandmother a white dress with embroidery all along the edges and insisted she attend the event. My grandmother refused. Being twenty-five years old at the time in 1953, she felt out of place and like an 'old maid'.

After some persuasion, my grandmother put on this special white long-sleeved mid-length dress and attended the festival with her girlfriends. Although she wanted nothing to do with strangers, that day, she met a traveling soldier, my grandfather, as pictured in figure four.

Figure 4. My grandparents upon meeting

The Creative Process Continuum

Eureka Moment

"We are Catholic. It's a sin. She wouldn't, never ever." ~Silvia Moreno-Garcia, *Mexican Gothic*, 152

Although I grew up in a very religious household, I often struggled with religion. My sister and I both attended a Jesuit high school, where many of the same values from my church-going childhood were reinforced upon us. We followed religion/religious classes and participated in weekly mass; I even had to partake in a week-long silent religious retreat. The retreat is one of the biggest secrets amongst the student body of my high school and is known as Kairos. Almost the entire senior class goes on this retreat in different groups throughout the year, keeping the secret that it is a silent retreat. Once the retreat is over, you are sworn to keep the secrets of the retreat a secret for the next group to experience the 'magic', as some might call it, of the trip.

On coming to college, I abandoned much of my faith. Despite my high school, experience, as I got older, the strictness or imposition of belief began to fade. During my senior year of high school, my mom got sick, which pushed both my parents back towards the church, while my sister and I became more distant from it. The more important events of dance practice and school work overtook family dinners and attendance at mass. While the ideals and values of the religion lingered, many of the acts were long gone.

Sitting around in my family home during the pandemic, I was reminded of many of these small religious moments. The baptisms, the confirmations, the enormous family brunch that occurred post mass. I re-discovered the many religious relics that remain in my family home–crosses, photos from various religious occasions, the rosary, and even the statue of Mother Mary in our garden all served as reminders of religion.

During this time at home, I picked up the novel

Mexican Gothic by Mexican-Canadian author Silvia Moreno Garcia. The story centers around a twenty-something girl growing up in a wealthy religious family in Mexico. She fears her cousin's life is in danger within her new marriage, taking the story to a small mining town in Mexico. The family did not believe in fantasy, but in faith. With vivid imaginative illustrations, the imagery all felt too similar. The yellow canaries singing in the gardens, the crosses on the wall, the figurines of saints, and the love of family all reminded me of my family and my cultural history.

The story is set in the 1950s, a time when many questioned race, ethics, morals, and religion, similar to the time of 2020. As someone of mixed race, I never really felt part of one race or culture more than another. My family followed many practices of the Mexican, Chinese, and Portuguese cultures. I know the languages, the foods, the countries, the special holidays. Yet, I would not necessarily identify with any or one specific culture. The one main connector between my family culture is the Catholic religion.

Upon arriving back at school, I decided to take a Latin American Art history class, where I fell in love with the history of Mexican culture. I saw so many connections between ancient Mexico and my life as a Mexican American. All of the imagery from the class and my childhood background came together to inform this larger picture of what my collection was to be.

Religion, race, art, culture, family, belief. I became so curious about what it all meant, what it all had to do with me. What was my family history, how does that intertwine with these beliefs, and where was the beauty in all of it? That is what my collection focuses on– finding the beauty in this long history of culture, art, and religion.

Breaking Down the Problem

Part 1: History Of Bridal Design

After working for various fashion companies, it became clear to me that there was a gap specifically in the bridal market. What is Bridal Design? Is it fashion designed for reality, based on fast fashion in the sense that those designs are being fit as a means to an end and mass-produced? Or is bridal design an art form that speaks to the fantasy of fashion design at the highest level of luxury and haute couture?

This collection questions where bridal design falls in the intersection of fast fashion and haute couture. The collection response will focus on the art and detail of bridal wear, with the goal of pushing the boundaries of "traditional" bridal wear. Incorporating wovens to create a remarkable ensemble, this collection will prove that bridal wear is an art form and worthy of fashion's critical taste.

Bridal wear, for too long, has been pushed aside by the fashion industry when it was once the pinnacle point of a fashion collection. Bridal wear often ended each runway show with the most dramatic moment, speaking to the connection between fashion and haute couture (Wischhover, 2014). "A moment of fantasy", as Carmela Spinelli, the chair of SCAD's fashion department described in an interview with writer Cheryl Wischhover for *Fashionista*.

Fashion needs that moment again; it needs the allure of glamour and awe. In this post-pandemic world, people are craving glamour and the need to dress up (Maguire & Binkley, 2021).

As the end of the pandemic allows for more events to occur, brides are being introduced to a new era of what a wedding is. Many have had to downscale or cancel their original plans over the last few years, leaving the idea of extravagance out (Kambhampaty, 2022). With over a year of Zoom events and ceremonies, bridal wear has changed with the times. My bride is one who is aware of the cultural and sustainable implications of our new world. However, my bride is a person who wants a 'fashion moment' after succumbing to many of the pandemic trends. The pandemic changed the whole process of bridal wear starting with how to shop for a bridal dress. This familial, special moment was taken from a lot of brides who had to choose their closest one to two people to shop with or were forced to pick a gown virtually via a zoom appointment. Many forwent even buying a dress starting separate trends of pantsuits, minidresses, or bridal separates. Looking toward the future, I aim in this collection to bring those moments of glamor back while still adhering to the trends of the current time. For years, the most esteemed fashion houses closed their runway shows with wedding gowns including Chanel, Guo Pei, Jean-Paul Gaultier, and many more as seen in figure five. However, in the last few years, fashion has discarded this tradition of the "last bride", either not showing a white gown at the end of the show or doing a completely separate show for bridal. Separating the two -- couture and bridal-- has created a gap and allowed the bridal design to fall out of fashion and into mass retailers. It is no longer necessary to create a 'fashion moment' as it was when bridal was included and recognized by the fashion industry as fashion. Brides today might buy a wedding gown from a catalog or online just as they would fast fashion. My goal is to bridge the gap of bridal within the fashion industry while finding beauty within my cultural background and history.

Part 2: What makes a fashion collection 'remarkable'

Remarkable, is defined by the Oxford dictionary as worthy of attention. For years fashion designers have created 'remarkable' collections. For example, Alessandro Michele's Spring/Summer 2019 collection for Gucci created both fashion and music history within the show with a performance by Jane Birkin. Kerby Jean-Raymond's Spring/Summer 2020 collection for Pyer Moss intertwined gospel, hip-hop, blues, and fashion. John Galliano's spring/summer 1998 extravaganza collection for Dior did so. All of them included at least these five aspects that I have defined as making a remarkable collection:

1. **Fit**: Fit plays an incredibly important role in a collection. Even oversize pieces have an aspect of fit. This is where scale comes into play, does a skirt overpower the bodice? Does the bodice overpower the skirt? Is it tight around the bust? Maybe the garment is tight around the waist? Or maybe the garment is not tight at all? How does it interact with a model? Can he/she/they move in the garment?
2. **Mystery**: How does the body play with the sense of mystery and wonder embedded in the garment? Are the eyes saying something behind a veil or headpiece? Are there any aspects of delicacy that keep an audience on their toes? How does material usage aid in the aspects of mystery?
3. **Emotion**: A collection should evoke some sort of emotion; what should the audience be feeling upon watching the collection? Is the collection conveying a story? In this case, is it one of family and cultural heritage? How should the model feel while wearing the garment? Is it a happy emotion? Stoic? Does the audience feel affected? How does texture play into this? How does the concept convey this?
4. **Fun**: Does the audience want to see more? Is it exciting? Are the shapes new or done differently?
5. **Cohesion**: Is there a sense of flow? Does it make sense with the concept? Is there a clear connection? If not, was that done purposely?

Conceptualize Design Ideas

Using all of my research and inspiration boards, I began my design process by sketching on a dress form as seen in figure six. This allowed me to conceptualize the front, side, and back of each look. I went through hundreds of sketches picking the top fifty, then the top twenty, then the top ten. I continuously edited drawings moving from drawing on dress forms to free-hand sketching. I started with an initial line-up all done by hand on the printed dress forms.

I found myself continuously drawing pieces with textured shapes. These ideas were among the first I explored in my design process. The design, a

Figure 5. Jean-Paul Gaultier Fall 2017 Couture; Chanel Fall 2012 Couture; Christian Lacroix Fall/Winter 2009

Figure 6. Initial Sketches

column dress with a textured top and sleeves, with a large cape in the back that fanned around the shoulders and neck, played with both scale and texture. In my next design, I continued to play with texture. I again started with a simple column dress and then began draping. I originally played around with cowl necks draping in both muslin and polyester silk. Due to a mistake of pinning, I landed on this interesting shape around the hips. The fabric moved with the body. I loved it. I tried recreating the shape in paper, muslin, and again with the silk. However, upon further analysis, the shape became more complicated to recreate and more difficult when I changed fabrics.

I moved back to working with cowl necks trying to recreate the aspects of movement and texture. I went back to designing a variety of cowl neck bodices. Cowl neck backs with boat neck fronts, front cowl necks with a back cowl neck and so on. I knew I wanted to design a suit in the collection and suddenly began playing with the idea of a cowl neck suit. I was curious on multiple levels about how to do this. The front needed structure to convey the shape of a suit and for the lapels, but the back needed drape for the cowl. I was intrigued. This design played with fit, scale, movement, and even texture. My initial pattern was simple– take the front and back of a jacket pattern, but replace the top back with a low draping cowl. Keeping all of the original darts of a jacket, I made the pattern in muslin. Then the problems arose. What material would speak to the whole design? Do the darts work? Is it fitting tightly enough around the waist to support the structure and drape?

I tackled each problem in a particular order. I started with the fit, as this would affect the darts. I played with cutting the fabric on the bias and added a yoke to support the cowl. Then I moved to the darts. This was a game of chess. Each new dart design affected the support of the cowl. I moved the original darts, to combine into a single bust dart and a fish eye dart. In the next rendition, I tried removing the bust dart completely to only have a fisheye dart. There continued to be slight fit problems around the curvature of the bust. I added the bust dart again, but this time curved the end of it so the dart would curve more around the bust. It worked. I then realized for the design and fit to fully work I could only have one front dart. I reworked the darts again for what felt like the fiftieth time. I kept the curvature of the bust dart but then shifted the bust dart to start closer to the waist to compensate for the excess caused by removing the fisheye dart. The final design contains an elongated front dart that fits perfectly around the chest and still supports the back. Due to this, the yoke's size and shapeshifted. The final yoke shape was a curved moon-like strip. This supported the cowl but allowed the jacket to sinch around the waist. I left the two back darts for a better fit around the hips.

This was the beginning of the draft of my final lineup. I included all of the exploration designs I had begun and developed other designs based on inspiration and how the collection was forming.

Initial Criticisms of Project

Analyzing my line-up I began hating the collection. Something felt like it was missing. I started disliking designs, finding them boring and not authentic to where I started. Discussing it with Erica Johns, Cornell's Fashion and Textile Librarian, the collection felt incohesive. One of my main goals was cohesion. I needed to go back to the drawing board. I needed to be reminded of the beauty, color, and design inspiration of the Mexican Catholic Church and my family heritage. I fell too far into the trends of bridal design, which was one of my goals to not do. I did not want to fall into the 'basic' bridal, I wanted this to be a fashion collection not a mass-market bridal collection for the basic bride.

Develop Ideas Further

I went back to my sketches and original ideas. I re-examined my inspiration slide decks. I began adding the accessories. I needed to see the full collection. This is when I began to cut ideas. I cut two of my original exploration ideas, building off them to create better designs. The original exploration did not feel like it fit with my original concepts, or with my collection's shift in direction.

I wanted the collection to focus on fit, movement, scale, and attention to detail to highlight the aspects of a strong collection– fit, mystery, emotion, fun, and cohesion. I finalized my collection to respond to each of these.

Figure 7. Final Lineup

5.1.7 Explain Ideations

Figure 8: Look One. After looking at so many aspects of the Catholic Church and Mexican Culture, the first thing I associate with the two is prayer. Inspired by the shapes of Catholic vestments and prayer shawls, I started creating shapes around the shoulders that prevented the shoulders from moving but allowed the arms to move specifically into a prayer position. I explored a few different shapes before deciding on the first rendition, a clean-cut top bodice.

Figure 9: Look Two. Straying from the traditional gown, I wanted to create a bridal suit inspired by the architecture of the cathedrals I looked at. After draping a suit jacket, it felt very stiff and masculine. I played around with the idea of a cowl neck back which posed a variety of problems. The front needed structure, but the back needed drape. It was the most difficult piece in the collection to make.

Figure 10: Look Three. In a lot of the inspiration I looked at, many of the saints, in particular Our Lady of Guadalupe, wore multiple layers often a jacket or shawl with a more fitted dress. This look is my interpretation of that with the fitted dress paired with an oversized jacket meant to wear off the shoulders. I noticed many of the images of Our Lady of Guadalupe were adorned with gold or rays of light. She was also often adorned in Milagro charms. I used Milagros, which are Mexican luck or protection charms, for embellishment on the dress. Each one was hand sewn on.

Figure 11: Look four. My grandmother's garden was filled with so many different fruits, vegetables, and flowers including marigolds. In Mexico, marigolds are used for a variety of things besides decoration. For the Day of the Dead holiday, marigold gold flowers are often found on ofrendas. It is said that the flower helps guide souls between the living and the dead. Looking at the flower I was inspired by the literal shape and created my own version. I played around with size, shape, and placement (reference Test Ideas in textiles/ material- swatches). I wanted the shape to feel natural and effortless, not prim and perfect like a Hallmark flower.

Figure 12: Look Five. I continued playing with the floral shapes but in a different fabric. The polyester mikado fabric created a fluffier shape than the silk did. I again played around with a few different shapes, exploring both literal floral shapes and more abstract shapes. I ended on a fuller floral shape for the final. I also played around with placement but liked the shape on the side. Although the waist is covered, the eye creates a waistline due to the cut of the dress.

Figure 13: Look Six. For the last look inspired by the marigold shape, I went back to using silk. The look is a mini skirt and crop top made of silk twill. This look broke up the collection in terms of length and scale. The silk charmeuse flower was attached to the right side of the chest with a strap on the left side. Similar to the silk flower in Look Four, but slightly different, this flower had strands of different lengths falling from it, some even longer than the length of the skirt. The veil paired with the look has a mix of floral shapes in white corded lace. The edge blusher is two roses facing each other, which is a symbol of mutual love and affection.

Figure 14: Look Seven. I call this dress the ‘angel’ dress because when the sleeves are puffed out correctly they mimic angel wings. With a corset and boning underneath, the sleeves are attached at the neckline until the underarm, where they then drape down and are attached at the back by the zipper. The skirt is a high-low skirt in the sense that it starts higher on the hip in the front and drops below the hips in the back, with more fullness in the front.

Figure 15: Look Eight. The last look in the collection honors the traditional bride with a large full skirt and empire waist. I choose not to do a dress with a waistline at the waist as it felt too ‘princess’ and ‘cupcake’ bride, which is what I wanted to stray from. Inspired by a 1745 oil painting, the Black Madonna Mexican painting also known as The Virgin of Guadalupe, the shape of the dress encapsulates the body. The empire waistline allows for fullness around the whole body and support around the chest, with a similar shape to the dress of the Madonna. A dress starting at the shoulders with the same fullness would not have had the same effect.

Style and Photography

The collection was presented at both the Cornell Fashion collection and within the Martha Van Rensselaer gallery space in the show “Behind the Collections: An Exhibit” open from May 1 to May 8, 2022.

Presentation One: Cornell Fashion Collective

Garments were styled with a variety of accessories including veils, earrings, and bracelets as seen in figure fifteen. Each girl’s hairstyle was inspired by different traditional styles of braids in Mexico with flowers incorporated into their looks. Their makeup was inspired by artist Frida Khalo, images of Selma Hayek in the film *Frida*, along with other Mexican iconography.

Presentation Two: Behind the Collections An Exhibit

The display was inspired by a variety of museum theories for best practices for the display of cultural objects. Although the collection includes a few physical cultural objects, it was fully inspired by the culture and all of the objects within that culture. On the wall behind the work, the frames are set up in a salon-style of exhibiting work as seen in figure sixteen. The salon-style was established by The Royal Academies of Art in France and England in the late eighteenth century. These men held a monopoly on what ‘good’ art was. (Presutti, 2022)

The wall style that is behind everything represents the colonial roots that led the Catholic Church to be in Mexico which created this beautiful culture. The first Catholic Cathedral in Mexico was originally the site for El Templo Mayor, a place of worship for the Aztecs in Tenochtitlan, modern-day Mexico City (Cohen-Aponte, 2021). The site was dedicated to the gods Tlaloc, god of rain, and Huitzilopochtli, god of sun and war also known as the Turquoise prince (Cohen-Aponte, 2021). The jewelry made for the collection was symbolistic to many of the Aztec gods and goddesses. The tablespace that holds the jewelry is pushed against the wall to represent an altar of sorts again to honor the original culture. The flowers on that table and around the space shift the time period and are representative of marigolds that are used for Day of the Dead altars. This celebration is a blend of Mesoamerican, European, and Spanish cultures, exactly like the Mexican Catholic culture.

Look One Look Two Look Three Look Four

Look Four Look Five Look Six Look Seven

Figure 16. Images from the Cornell Fashion Collective Annual Show 2022

Left side of the Gallery

Close up of the Salon Wall

Part of the Wall Side

Figure 17. Photos of the Gallery

Conclusion

Through my collection I hope I was able to shine a light on a beautiful culture and tell my family's story, one that took me a long time to acknowledge and fully understand. There is still so much work that needs to be done with cultural representation in fashion, but I hope that this project was my start in creating my space of representation in the fashion industry. I hope that it was also the start of bridging fashion and bridal back together, creating moments of glamour and awe.

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Appendices

Seminar One: "Fragile Knowledge: Owning the Scars of Second-Generation Holocaust Survivors in Latin America" by Annette Levine, LACS Seminar Series" (February 14, 2022)

Identity can be a difficult concept. What does one do when their identity has to be hid or changed drastically? It was interesting to hear the family stories of second-generation Holocaust survivors who struggled with their identity after moving to a variety of Latin American countries. The talk discussed the dynamics of religion, identity, and family all topics similar to my work. The speaker brought up the concept of known vs not known vs kept a secret. I felt like this particular concept was very relatable as in any family there are things that are unknown and things that are secrets. Both are unknown but one is purposefully unknown. The talk also connected to my research of how culture and religion can be intertwined and sometimes difficult to do so. Many of the guests at the event talked about how they struggled to fit in as Jewish in places that were predominantly not Jewish and even in the same towns with Natzis in hiding.

Seminar two: Beyond Waste | Keynote Speaker | Colonialism, Climate Change, Consumerism, and the Need for Collective Change with Aja Barber (Feb 24, 2022)

Aja Barber was so fascinating to listen to regarding her thoughts on climate change, consumerism, and collective change. However, I found most interesting her thoughts on colonialism. This relates directly to my research as the Catholic Church only got to Mexico due to colonialism and the conquistadores. In terms of sustainability, Barber discussed how so much excess often ends in foreign countries that are home to predominantly black and brown people. Another example she discussed regarding fashion and colonialism was the role of India and the British. One of the many reasons England colonized India was to cut off trade routes of the numerous fashion products India was creating – cotton, silk, dyes, pigments, etc. The other aspect of her seminar that I felt was very related to my project was the conversation on cultural appropriation vs appreciation. This was an aspect I myself struggled with, as someone who is Mexican how do I appreciate this culture within a collection without appropriating it. I really tried to show the beauty of a culture within the collection without overstepping. Lastly, she discussed how fashion can institute positive change for black and brown communities. I truly hoped that people looked at my collection and saw the beauty of Mexican culture and hope that any child of that culture can see themselves in my garments and within the fashion industry.

Seminar Three: Sustainable Systems for Fashion: Biomimicry and the Future of Fabrics by Tricia Langman (March 25, 2022)

Tricia Langmans discussed so much about sustainable systems for fashion within her talk, particularly about the future of fabrics. She reminded viewers of the importance of biomimicry and how we have to think sustainable. We are out of time to do otherwise. Thinking from the perspective of the Bridal industry, it's fascinating to me how sustainability will be further implicated. At the root of bridal fashion is the notion of people needing a dress or dresses for a single day or maybe a weekend. Then that dress gets preserved and possibly passed to the next generation or lands in waste. It's the exact opposite of sustainability. Therefore Langmans talk about the future of fabrics was particularly interesting to me. Can these bridal dresses and fabrics be reused for other things? The answer is yes, but that other thing has yet to be figured out.

Seminar Four: "Sandy Rodriguez Final Keynote Presentation" (April 28, 2022)

Sandy Rodriguez discussed her work with the Florentine Codex, a book I studied almost a year ago when learning about Mexican history and Mexican art history. The book inspired some of the original shapes I was creating, especially the flora and fauna. It was an incredible talk to hear as my project was wrapping up and came full circle from one of its original inspirations.

With a background in museums, she was also a great resource for discussing the placement of cultural objects within a space to both respect and acknowledge their origins.

Definitions

The modern trend of wearing white to one's wedding began in 1840 when Queen Victoria wed Prince Albert. Prior to this brides often wore their most expensive dress which was often of a dark color so that it would not dirty as easily as white would. The white trend has stayed fairly steady over the last century with few expectations, one being the period of World War II. Due to fabric shortages, some brides resorted to creating gowns out of their future husband's parachutes (Yellin). However, in the United States, the American Association of Bridal Manufacturers lobbied against fabric shortages. They stated in 1943, "American boys are going off to war and what are they fighting for except the privilege of getting married in a traditional way? They're fighting for our way of life, and this is part of our way of life" (Schoeny).

I chose to stick mostly to the white color family including some bright whites, and ivories for the collection.

Figure 11. Color Inspiration: Queen Victoria's wedding dress

I had a difficult time choosing to just do white looks, as I felt that the Mexican Catholic culture is so colorful and full of life while white symbolizes prosperity and virginity. White felt stiff in comparison to the fluid colors I felt were representative of Mexican culture. I choose to incorporate color through accessories and styling choices so I could incorporate my inspiration from the Mexican Catholic culture while still incorporating some aspects of traditional bridal wear.

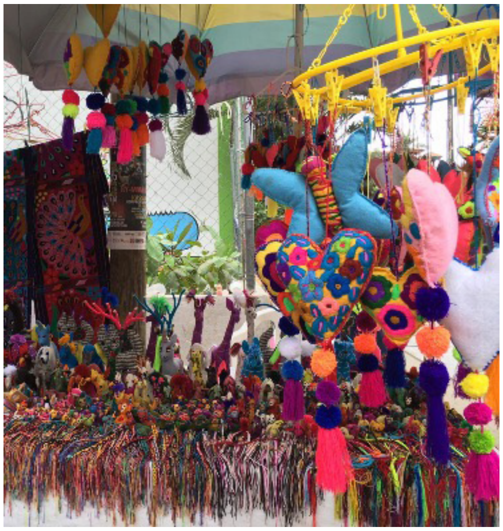


Figure 12. Color Inspiration: photo of a table in Sayulita, MX and color inspiration

Figure 13. Use of colors in accessories

Figure 14. Colors in Jewelry

Color Story

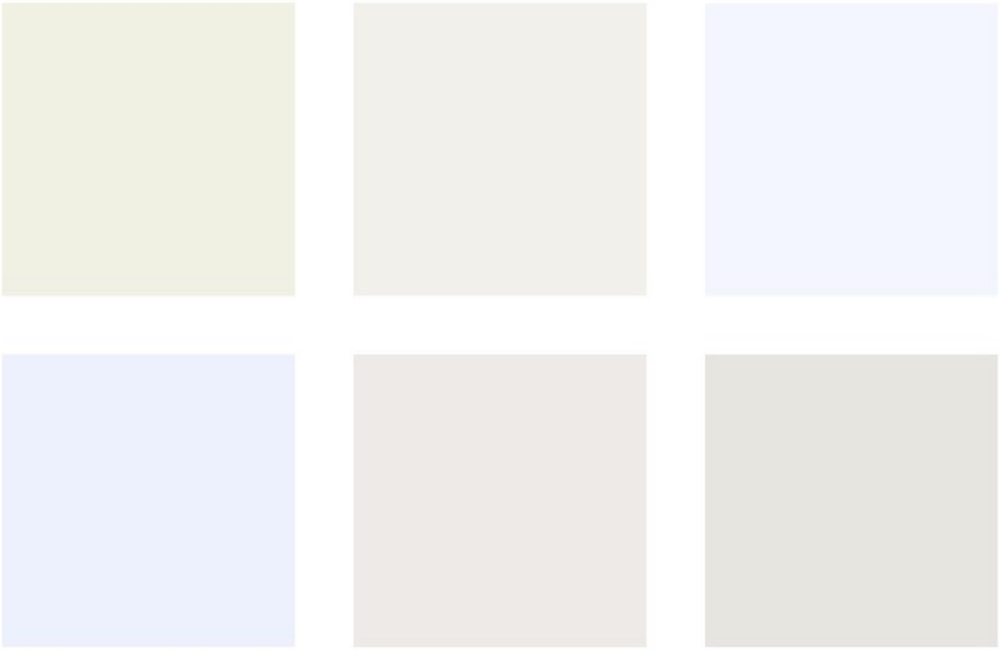


Chart 1. The color palette is inspired by traditional bridal wear, and the colors of Mexico and Cathedrals

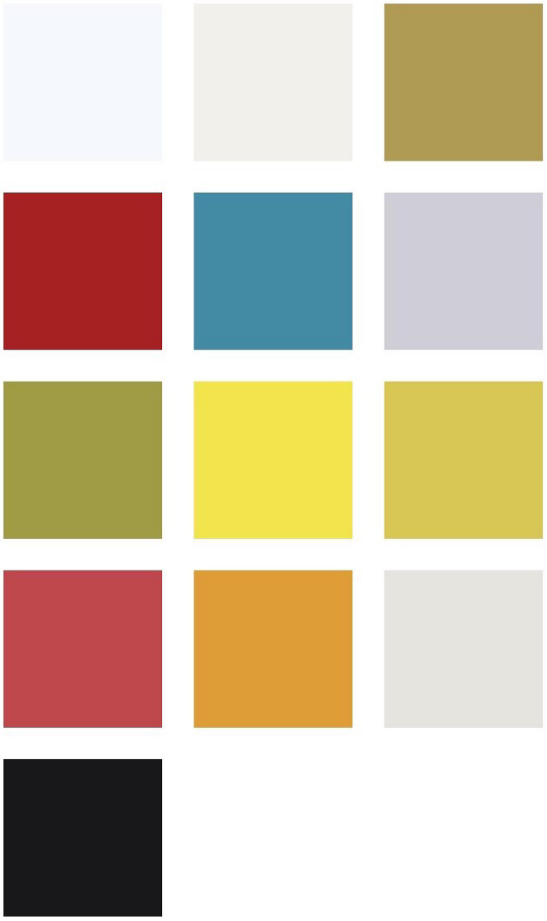
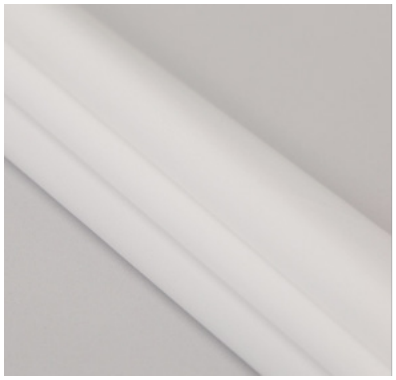


Chart 2. Pantone Colors for Accessories including veils and jewelry

Fabric Selection



Double Faced Polyester
Georgette

Mood Fabrics $7 a yard
Gul Ahmed Fabrics INC $6 a yard

Polyester Silk Mikado
Exclusive Fabrics
$10 a yard

Ivory Silk Charmeuse
Mood Fabrics $37

White Silk Charmeuse
Mood Fabrics $37

Ivory Silk Taffeta
Mood Fabrics
$32 a yard

Silk Twill
Mood Fabrics
$18 a yard

Ivory and Gold Floral Lace
Britex Fabrics
$275 a yard

White Corded Lace
Britex Fabrics
$55 a yard

Ivory Tulle
Britex Fabrics
$3.49

Chart 3. Fabrics used in Final Collection


White Tulle
Britex Fabrics
$3.49


Soft Tulle
B& J Fabrics
$33 a yard


Mesh
Spandex World
$6 a yard


Silk Lining
New Star Fabrics INC $13 a yard

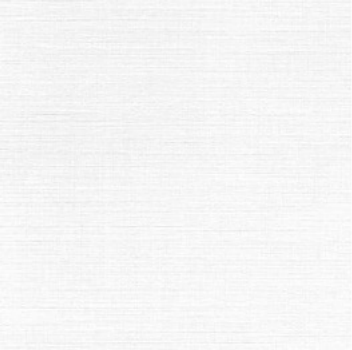

White Linen
Joanns Fabrics
$9.99 a yard

Chart 3. Continued

Accessories

Veils

Over the summer of 2021, I learned the couture techniques of veil making from both Cornell Alumna and Creative Director of Monvieve, Allison Miller, and the Creative Director of Sarah Nouri, Sarah Nouri. I used these skills and applied them in my collection and process of veil making.

The first veil I worked on is similar in length to a chapel veil at two and a half feet long and ~106 inches wide. I started by cutting the tulle. I knew I wanted this veil to be a mantilla veil so I cut the base wider than the top and eliminated the length for a blusher. Next was the lace. I spent months researching lace: where I wanted to get it, how I would cut it, and most importantly how I would organize it. I did veil studies creating designs with paper before purchasing anything.

During winter break, I picked out three different laces in varied white tones. A bright white Chantilly lace, an ivory corded lace, and an ivory Chantilly lace corded with gold. I chose the latter for the mantilla veil. I carefully hand-cut each piece of lace and laid it out on the tulle playing with different designs. Once I finalized the design I liked, I hand pinned each piece of lace onto the cut tulle then hand stitched the lace.

The second veil I designed is similar in length to the first veil, however, it also includes a thirty-two-inch blusher. I used a white floral corded lace that outlined the edges of the tulle.

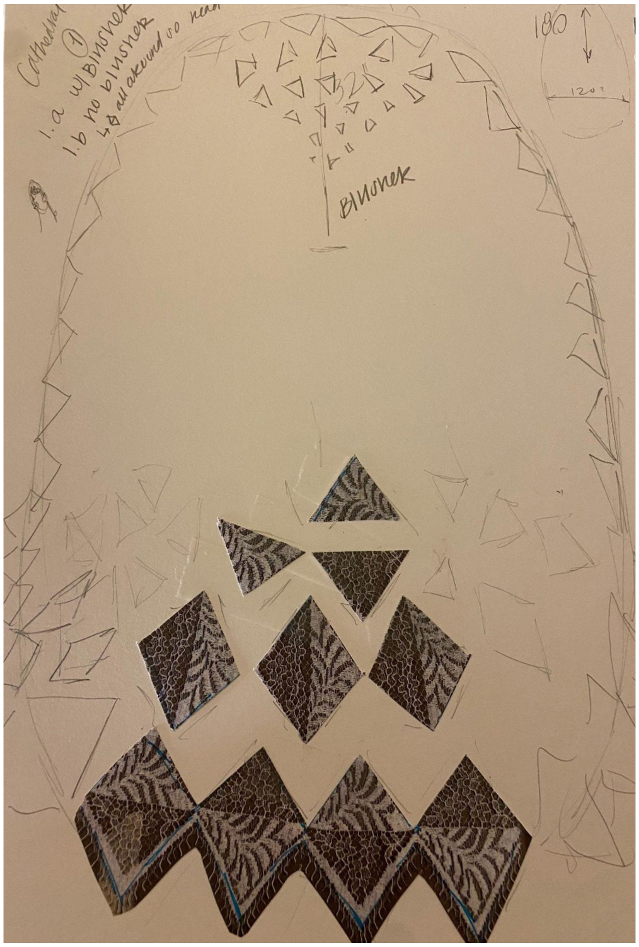


Figure 38. Veil Study

Figure 41. Veil Three behind Veil One for comparison

Figure 39. Veil One

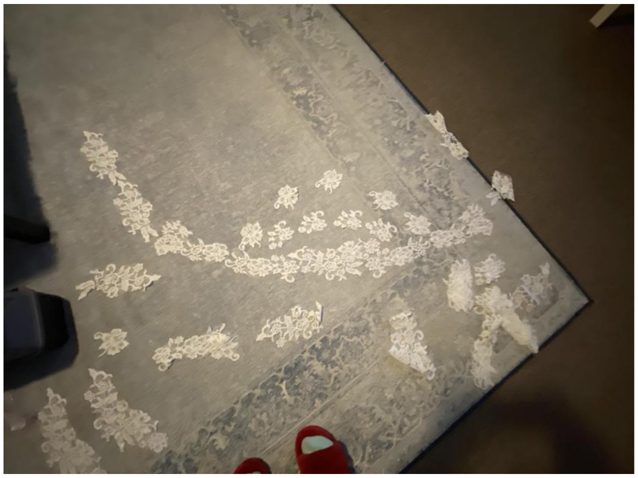


Figure 40. Veil Two

The third veil in the collection was the longest of them all. It was about five yards long, 110 inches wide at the front with a 34-inch blusher, and 120 inches wide at the end. It was made with an English tulle, so unlike the two veils with lace, it is thicker and less opaque in color.

Jewelery

The jewelry of the collection was inspired by a mix of Gods and Goddesses from Teotihuacan and from jewelry of the Catholic Church, particularly rosaries. I took the most inspiration from the supreme deity of Teotihuacan, the Great Goddess. Her statue was found near the pyramid of the moon and a print of the Goddess was rediscovered in 1890. She was associated with water, caves, and fertility. She later became a symbol of national identity and pride. Images of the Goddess were found all over in different forms– stone statues, paintings, carvings, drawings, etc. The Goddess was distinguished by a few different traits, including her cleft head, which was a reference to the mountains; the symmetry of her body and face; and her headdress. However most notable were her ear spools, almond-shaped eyes, and arms decorated with bracelets. (Cohen-Aponte)

Figure 42. Great Goddess Statue

Figure 43. Great Goddess Print

Figure 44. Jewelry Process three

Figure 45. Jewelry Process Two

The circular shapes of the beads I chose was inspired by the Great Goddess ear spools. I played around with symmetry making many of the earrings asymmetrical in both shape and length.

The bracelets were both inspired by the Great Goddess and the rosary.

Figure 46. Example of Jewelry on

Figure 47. Sample of bracelets

Authors' Biographies

Alicia Chung, Ed.D. is an Assistant Professor of Population Health at NYU Grossman School of Medicine. Her research interests include health promotion of sleep, diet and play behaviors that reduce obesity risk in families of color to narrow the health disparities gap. In her current role, as a faculty member in the Center for Early Childhood Health and Development (CEHD), under the mentorship of Laurie Brotman, PhD (CEHD, Director), in the Department of Population Health, she leads the community engagement partnership for a place-based initiative to enhance school readiness among children, families and school leaders in Sunset Park, Brooklyn. Through this work, in collaboration with colleagues at CEHD, she is able to culturally adapt parent-focused interventions, to address the social-emotional wellbeing of Pre-K children. Working alongside NYU's Family Health Center, she is able to develop cross-collaborations in the medical, education and community setting to develop strategies to address social determinant of health needs for children and families.

Dr. Chung's research interests also include the utility of digital technology tools, such as social media, mobile apps and wearables, to engage hard to reach populations in health behavior change. She has developed and lead several pilot initiatives that promote sleep, diet and physical activity via e-health

Juliana daRoza is a recent graduate of Cornell with a degree in Fiber Science and Apparel Design. While at Cornell she also pursued a minor in art history and human development. Since graduating, she is currently working in design in New York City. Her work is constantly inspired by her surroundings, history, and life.

Bryce Demopoulos is a senior in CALS studying biological engineering and astronomy. His interest in virology hails from high school, and he has also pursued other avenues of biological research at the Hospital for Special Surgery and Weill Cornell Medicine. Bryce hopes to attend medical school next year, where he can continue to pursue his dual passions for healthcare and research. Outside of academics, Bryce volunteers with the Red Cross and the Cornell Food Pantry, and he can often be found snowboarding, running, or playing lacrosse with the respective club teams at Cornell. He also loves getting outside for a hike, a swim at a gorge, or a friendly (or competitive) round of Spikeball.

Leone Farquharson is an award-winning photographer and visual artist with a passion for social impact creative work. She is a globe trotter as she was born and raised in Kingston, Jamaica but lived in Ontario, Canada for some of her childhood and attended university at Cornell University in Ithaca, New York.

Aside from her creations, she is a proud athlete and NCAA All-American in the Long Jump with a budding passion for mental health advocacy in sports. In 2021, she won the Theodata Ladas Award for the best jumper (among high jumpers, long jumpers, pole vaulters and triple jumpers). She was involved in leadership throughout the extended campus community.

Moreover, she attained a B.A. in Biology and Society with a minor in Inequality Studies on the Health Equity track. She hopes to encourage the 'multipotentialite' career dream which balances global health initiatives for lower socioeconomic groups (such as BIPOC specifically women's health), mentoring young students and athletes, social entrepreneurship and digital health. She is a budding businesswoman with massive vision and ideas always on the horizon. Her future projects hope to create value-based precision medicine technologies and innovations. She will be a change-maker in her scopes of interest. Current publications include Health Disparities Research in Pediatric Sleep Apnea!

Sarah Honaker, PhD, is an Associate Professor of Pediatrics at the Indiana University School of Medicine, and the director of Behavioral Sleep Medicine at Riley Children's Hospital. A clinical psychologist with board certification in behavioral sleep medicine, she provides treatment for children with insomnia, circadian rhythm disruption, and other sleep disorders. Dr. Honaker also directs the Healthy Sleep for Kids research laboratory and conducts research on sleep in children and adolescents. She currently holds research funding from the NIH, CDC, PCORI, and the American Academy of Sleep Medicine Foundation, and has more than 30 publications on pediatric sleep.

Ethan Lin is a student at the University of Wisconsin-Madison majoring in microbiology. He is currently involved in a developing a high throughput model for studying therapeutic response to ampullary cancers. Outside of school he is an avid football fan and plays a variety of brass instruments.

Angel Nugroho is a recent graduate and double majored in Archaeology and Information Science. Currently, she is working as a technology consultant for the public sector. At Cornell, she was part of the Humanities Scholars Program where she completed this manuscript with advisor Maia Dedrick. She also was also a member of Women in Computing at Cornell and Early College Awareness. In the future, Angel hopes to pursue graduate school and continue learning about the world of conspiracy and disinformation.

Sarah Rackowski is a Cornell CALS Sophomore majoring in Environment and Sustainability. She began this Research in High School, and has continued studying vertebrae biology at the Cornell Museum of Vertebrates. Sarah's main research interests include the biology and ecology of invasive and introduced species and speciation and diversification on short time scales.

Dr. Manu Ramalho has a Ph.D. in Molecular and Cell Biology from UNESP (Brazil). She did her Postdoctoral Researcher at Cornell University (Ithaca, NY, USA) in the Moreau Lab (Dr. Corrie S. Moreau). Currently, she is Assistant Professor of Department of Biology at West Chester University (PA). Her research focuses on understanding the mechanisms that impact microbial communities, unraveling the role of ecology, diet, behavior, stage of development, and also phylogeny of the host in these symbiotic interactions. To better understand these mechanisms, she uses ants as a study model.

"In several ant genera, symbiotic interactions with microbial communities have been shown to have profound impacts on the host. But more than that, ants can be found across the globe and have an immense diversity of behaviors and ecology. Also, ants are fascinating!"

Steven Wang is a recently graduated Entomology major in CALS studying ant-microbial interactions. His research interests include phylogenetic reconstruction, insect-microbial interactions, and invasive species spread. He hopes to pursue a PhD focused with myrmecology, in a way that combines both fieldwork and wet lab experience. In his free time he enjoys hiking, birding, uploading and curating on citizen science sites like iNaturalist and Bugguide, and macrophotography. He is originally from Houston, where you can sometimes spot him roaming the Katy Prairie and at UV sheets in the woods.

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