Title
Deciphering The Ecological Impact Of The Passenger Pigeon: A Synthesis Of Paleogenetics, Paleoecology, Morphology, And Physiology

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DECIPHERING THE ECOLOGICAL IMPACT OF THE PASSENGER PIGEON: A SYNTHESIS OF PALEOGENETICS, PALEOECOLOGY, MORPHOLOGY, AND PHYSIOLOGY

A thesis submitted in partial satisfaction
Of the requirements for the degree of

MASTER OF ARTS

in

ECOLOGY AND EVOLUTIONARY BIOLOGY

by

Ben J. Novak

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Abstract

Deciphering the Ecological Impact of the Passenger Pigeon: a Synthesis of Paleogenetics, Paleoecology, Morhpology, and Physiology

Ben J. Novak

The extinction of the passenger pigeon may have long-term consequences to eastern North American forest ecosystems; however, the past and ongoing consequences of the species' extinction cannot be understood nor predicted without thorough knowledge of the species' historic impacts. According to historic accounts, in abundance passenger pigeons generated large-scale understory and canopy disturbances. Key components needed to fully understand the impact of these disturbances remain contentious without additional data. To produce necessary data, the recent population history of the species was reconstructed using 41 complete mitochondrial genomes; limitations of diet were assessed by modeling oral gape size and the effects of digestion on seed dispersal ability was experimentally analyzed using living band-tailed pigeons, *Patagioenas fasciata*. Population genetic modeling found long-term stable abundance of the species over the past 20,000 years, during which time forest communities continually changed, indicating that passenger pigeons were ecologically resilient. The gape size of the passenger pigeon presented limitations to consuming the largest seeds of the Northern Red Oak and the American chestnut while exhibiting no limitations to consuming acorns of the white oak family: presenting differential selection pressures to various tree species. Passenger pigeons were not fecal dispersers of seeds, precluding mutualistic coevolution with mast bearing trees. When examining the native communities of eastern North America, disturbance dependent plant and animal species predominate, which I propose is the result of long-term impacts of large passenger pigeon flocks.

Dedication and Acknowledgments

I am indebted to Dr. Beth Shapiro for accommodating my odd disposition into the community of her laboratory. As my advisor she simultaneously kept my work grounded in pragmatic science yet indulged my unorthodox demeanor.

I dedicate my work to my grandfather, Anton Jacob Novak, Jr. (1928-2011), whose love of birds and endless curiosity of the world around him cultivated my passions in science and nature; to my parents, who have unceasingly supported my endeavors through my best and worst times; to my entire family for their encouragement and love; to my friends who have always kept my feet on the ground and my head fitting through a standard doorway; and finally to my beautiful wife, Erika Novak, who not only entertained my eccentric notions, but also used her legitimate occupation (Doctor of Veterinary Medicine) to assist my work and fund my tuition.

This project would not have been possible without myriad contributions from many people. All of my colleagues at the UCSC Paleogenomics Laboratory gave insights that shaped my thinking on this subject. Peter D. Heintzman assisted in some early laboratory preparations, Dan Chang analyzed mitochondrial genome datasets in Arlequin v3.5, and André Elias Rodgrigues Soares conducted the majority of bioinformatics work from which I've drawn conclusions. My friend Ian Roderer has edited my writing for over ten years, and graciously ensured grammatical and rational sanity to the early drafts of this thesis. I thank James Estes, Steven Apfelbaum, Ryan Phelan, and Stewart Brand for their guidance. Sal Alvarez and Paul Marini for their education on pigeon care. I thank Allan J. Baker and Mark Peck of the Royal Ontario Museum; Paul Sweet and Thomas J. Trombone of the American Museum of Natural History; Kathryn Murano Santos and George McIntosh of the Rochester Museum & Science Center; John Demboski and Andrew Doll of the Denver Museum of Nature & Science; David Willard of the Chicago Field Museum of Natural History; and Gregory Sohrweide and Marie Lorraine Pipes (independent archaeologists) for their roles in providing tissue and bone samples of passenger pigeons for DNA sequencing. Peter Capainolo, of the American Museum of Natural History, and Brian Schmidt of the Smithsonian National Museum of Natural History, provided skull measurements of passenger pigeons. Carla Cicero permitted access to research specimens at the Museum of Vertebrate Zoology of Berkeley. Access to extant columbid cadavers was supervised by Krysta Rogers of the California State Fish and Wildlife Department. Holland E. Shaw, Joseph Richinelli, John Bender, Gina Gilliland, Don Sparks, Gayle Brillhart, Bill Sayers, and Jeff and Cheryl Carpenter collected mast crop samples. Funding for DNA sequencing came from various grants of the UCSC Paleogenomics lab with private contributions from James Sartor, Sarahí Avelar Aguiñaga, Janette and Anton J. Novak Jr., Walta and Timothy J. Novak, Marietta Koppang, Carmen and Nathon Maxwell. Funding for my labor during this work was provided by the Founding Funders, Angel Funders, and many supporters of Revive & Restore (http://longnow.org/revive/our-supporters/).

Section 1 Thesis Introduction

The history of the passenger pigeon, *Ectopistes migratorius*, has garnered a great deal of attention in academia and public media since the turn of the 20th century. This great attention stems not from the species well known historic abundance, but from how swiftly billions of birds declined to extinction. The species likely numbered in the billions as late as the 1870's, but by the 1880's only thousands survived. In 1898 a mere 25 records exist reporting sightings or collection of the birds in the wild. The last bird shot in the wild was collected in 1902 (J. Greenberg 2014). While the species declined for over 30 years, the bottleneck of the passenger pigeon was exponentially precipitous over a period of less than 5 years from autumn, 1878 to spring, 1884.

Early explanations of the birds disappearance tried to dismiss the role of commercial food harvesting as a causative agent of extinction, but later evaluations consider human harvest and human caused deforestation to be the major, and likely sole, factors contributing the species rapid demise (Schorger 1955; Stanton 2014; Temple, Zuckerberg, and Stanton 2014). Early recognition of man's involvement in the bird's extinction was used as a rallying point for the creation of some of the first modern conservation laws (J. Greenberg 2014). The undeniable involvement of humans in the extinction of a wild species was a turning point in global paradigms towards natural resources, ushering in the modern conservation movement as we know it today.

The species has entered the academic and media spotlight more intensely in recent years following the 2013 announcement of a project planning to use modern genetic engineering technologies to recreate the bird and then reintroduce the species to the wild. This project, "The Great Passenger Pigeon Comeback", is directed by Revive & Restore within the Long Now Foundation, and brought me on board to lead the effort. The concept of recreating an extinct species has been dubbed "de-extinction" by popular media, a term adopted by academics.

The success of such an endeavor will rest heavily on a thorough understanding of the passenger pigeon's ecology, how the species' former range has changed since extinction, and how the project is defined. The de-extinction of the passenger pigeon is by definition the facilitated adaptation of an extant pigeon species, in this case the genomic editing of the band-tailed pigeon, *Patagioenas fasciata*, to serve as a suitable ecological replacement. The birds used for ecological replacement will possess passenger pigeon alleles for key traits influencing the extinct species' ecology. Ecological replacement is not a new means of conservation practice, and has been performed internationally several times (P. J. Seddon et al. 2014). Under United States Endangered Species Act proposed policies, such birds would qualify as "intercross" individuals (any individual resulting from the interbreeding or other genetic exchange of two species, USFWS 2000) of the band-tailed pigeon to be used for "non-native" introduction (Camacho 2015), though other legal interpretations may be assumed. However, it is important to note that current potentially applicable policies largely relate to endangered and captive managed species, of which the band-tailed pigeon is not. The band-tailed pigeon is classified as a migratory game bird for regulatory purposes.

What is largely agreed upon is that the introduction, or re-introduction, of wood pigeons (of which band-tailed pigeons and passenger pigeons are ecologically categorized) to the eastern United States fall into the category of translocation efforts with research and regulatory protocol precedence (Philip J. Seddon, Moehrenschlager, and Ewen 2014; Jorgensen 2013). Such precedence, and any future developments specific to biotechnology based ecological replacements, will require thorough risk assessments for both the species being introduced and the habitat designated for introduction (Philip J. Seddon, Moehrenschlager, and Ewen 2014; Camacho 2015). Considering the aspects required for reintroduction assessments (Philip J Seddon, Armstrong, and Maloney 2007) the ecological niche of this iconic extinct species needs to accurately characterized, which prompted my thesis study.

The ecology of the passenger pigeon has been widely, and often conflictingly, speculated among researchers and historians over the past century, almost exclusively in regard to the species' rapid extinction (Bucher 1992; Hung et al. 2014; D.E. Blockstein and Tordoff 1985), which is unsurprising given the extinction event is the most notable aspect of the bird's natural history. Though the primary focus of past research has aimed at explaining extinction, the one thing agreed upon by all researches is that the shear abundance of the species in historic times made their flocks an ecological force in nature. Considerations of how this abundance shaped the species ecological role in the environment have been remiss because the persistence of their abundance in nature has been a point of argument. The assumptions of some studies rely upon long term abundance of the species (Webb 1986; Ellsworth and McComb 2003), though it has been proposed that the species' abundance is a recent phenomenon stemming from Amerindian driven factors in the past 1,000 years (Jackson 2005) or more provocatively from Euro-colonial changes to the environment over the past 500 years (Neumann 1985). Recent population models derived from full nuclear genome sequencing report that passenger pigeons fluctuated between periods of abundance and rarity (Hung et al. 2014), however these population models are not informative for recent history (the past 20,000 years) from which changes in passenger pigeon habitats can be compared. Compounding the matter, recent unpublished analyses of whole passenger pigeon genomes from the UCSC Paleogenomics Laboratory reveals that the oscillations reported previously are not biologically real, but a modeling artifact resulting from the distribution of genetic diversity split between regions of low and high recombination throughout the genome. New data is needed to assess the population history of passenger pigeon abundance.

Some aspects of the passenger pigeon's natural history, which are not debated, come from firsthand historic observations. The species ate a variety of seeds and fruits from over 42 genera of woodland plant taxa (J. Greenberg 2014) with emphasis on tree mast and seed crops (oak acorns, beechnut, maple seeds, pine seeds, etc.) The species' dense colonial roosting and nesting flocks were observed to cause major forest disturbances to the canopy via the breaking of branches from overcrowded perching and to the understory via deposition of vast amounts of guano. It can reasonably assumed, given the size and density of passenger pigeon flocks, that when abundant, the birds were major competitors for food resources throughout their large foraging areas surrounding roosting/nesting sites (several hundred square kilometers, Schorger 1955). Potential competition with mice for acorns has led to the hypothesis that passenger pigeons played a key role in suppressing outbreaks of Lyme disease (David E. Blockstein 1998). The birds must also have been a major transporter of nutrients: consuming mast crops from the large foraging area and concentrating guano at the small roost/nesting colony (averaging 77.69 square kilometers , Schorger 1955). While no one has examined the potential impacts of passenger pigeon movements on nutrient transport, Ellsworth and Mccomb (2003) did attempt to model aspects of how the concentration of passenger pigeon guano impacted habitat. They predicted that the build up of guano would exacerbate the frequency of wild fires when combined with the physical damage caused by roosting/nesting pigeons, though their models did not provide significant support for the idea. The work relied on models of physical damage that were inflated compared to observation. Contrary to Ellsworth's supposition that passenger pigeon activity increased the likelihood of fires, historically fire frequency actually increased during and after the passenger pigeon bottleneck to extinction; fires decreased in frequency when actively suppressed by mid-twentieth century forestry management (McEwan, Dyer, and Pederson 2011). It should also be noted that the study used the chemical composition and deposition rate of red-winged black birds, *Agelaius phoeniceus*. Red-winged black birds are incapable of ingesting acorns (due to acorn size) and consume large quantities of insects – a diet not analogous to the passenger pigeon. The impacts of guano deposition suggested by the study took inference from observations of marine and wetland bird species, once again not analogous to passenger pigeon habitat use and ecology.

What has been assumed about passenger pigeon ecology without empirical evidence depends on the publications one reads; however two points have been largely agreed upon: 1) passenger pigeons preferentially consumed acorns, perhaps to the point of being dependent on oak resources, and 2) passenger pigeons consumed mast crops in entirety regardless of the seed species eaten. These assumptions have footing in historic accounts and data, but have not been analytically scrutinized by any means. The major point of conflict regarding passenger pigeon ecology is whether or not the birds were dispersers of seeds or predators of seeds: to date it is unknown if wood pigeons destroy seeds or not during digestion (ecological analogs being the band-tailed pigeon, and European wood pigeon, *Columba palumbus*).

The assumptions outlined above need to be evaluated with new data in order to understand the ecological niche and impacts of the passenger pigeon. The key missing information is population history, ecological physiology, and physical constraints to ecology. The open questions which can be addressed are 1) population history – what is the relationship of passenger pigeon population trends and habitat change, 2) physical – were there limitations to its diet, and 3)? physiological – were passenger pigeons seed dispersers or seed predators?

Section 2 Characterizing the Historic Niche of the Passenger Pigeon

Section 2.1 Introduction

Despite over a century of publications, the ecology of the passenger pigeon, *Ectopistes migratorius*, is still a speculative topic operating more so on independent assumptions than a consilience of available and producible data. The one thing that all publications seem to agree upon was that the species undoubtedly had a significant ecological impact resulting from its colossal abundance (3-5 billion) and high population density (flocks of millions to billions). However, despite that universal agreement, research has focused almost exclusively as to how the passenger pigeon's population ecology/biology contributed to its swift extinction with almost no focus at all regarding how that ecology shaped or was shaped by the species' former ecosystem. It is unsurprising then that even less research has considered the ecological implications of the birds' absence from eastern American forests over the past century. If the species indeed had a significant impact on the ecosystem, then 1) what was that impact and 2) can the effects of losing that impact be inferred?

What is definitively known of the passenger pigeon's ecology is derived from historical accounts and data. Flocks moved nomadically foraging for food throughout forests east of the Missouri and Mississippi rivers (figure 4). Fossil evidence indicates that the species was present throughout this range consistently throughout the late Pleistocene and Holocene (figure 1B). Their presence at any given roosting/nesting site was ephemeral but left long lasting successional effects induced by the severe forest disturbances they generated. The flocks produced both canopy and understory disturbances: thinning the canopy by breaking branches and trees by shear weight when overcrowding perches and depositing such a large amounts of concentrated guano as to clear the undergrowth completely (Schorger 1955). The passenger pigeon was observed to eat seeds and fruits of 42 genera of plants (J. Greenberg 2014), though it is regarded to primarily use mast crops of beech, *Fagus grandifolia*, and oaks (*Quercus* sp.) (Schorger 1955), assumptions supported by crop and stomach contents of birds collected from the wild (Cottam and Knappen 1939).

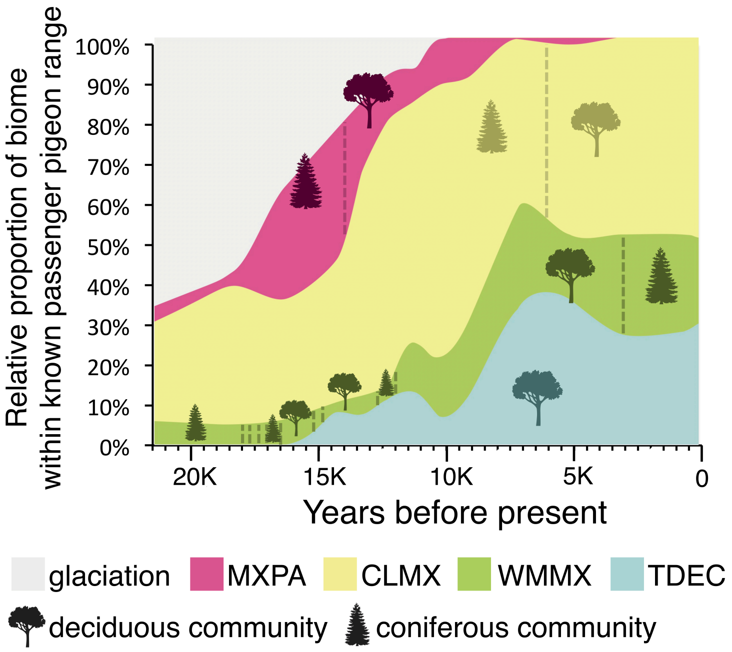
What is not known about the passenger pigeon is how the species' physiology, morphology, and population history shape its ecology with respect to the tree species with which it interacted. It is known that gape size is a limiting factor for avian diets (Wheelwright 1985), yet no limitations on passenger pigeon diet have ever been considered. Passenger pigeons have been assumed by some to be seed dispersers (Webb 1986; Aizen and Patterson 1990) and by others to be seed predators (Ellsworth and McComb 2003; Janzen 1971). To date no experiments have been conducted to evaluate seed dispersal or predation in wood pigeons with analogous diets to passenger pigeons (e.g. the band-tailed pigeon, *Patagioenas fasciata*, or European wood pigeon, *Columba palumbus*) (Webb 1986).

How forest biomes changed within the known range of the passenger pigeon over the past 21,000 years can be surmised from palynological data (figure 1A). When comparing changes in forest biomes to the passenger pigeon fossil/bone assemblages it can be deduced that the pigeons used the same geographic regions before and after major forest community changes, but how population abundance responded to these changes cannot be extrapolated from bone assemblages. The only way to assess long-term population fluctuations of the passenger pigeon must be inferred from effective population sizes derived from population genomics as was presented by Hung et al. (2014). The data presented by Hung et al., however, does not contain information regarding the past 21,000 years for which forest palynology data exists, a problem common to extrapolating recent demography from nuclear genomes (Li and Durbin 2011).

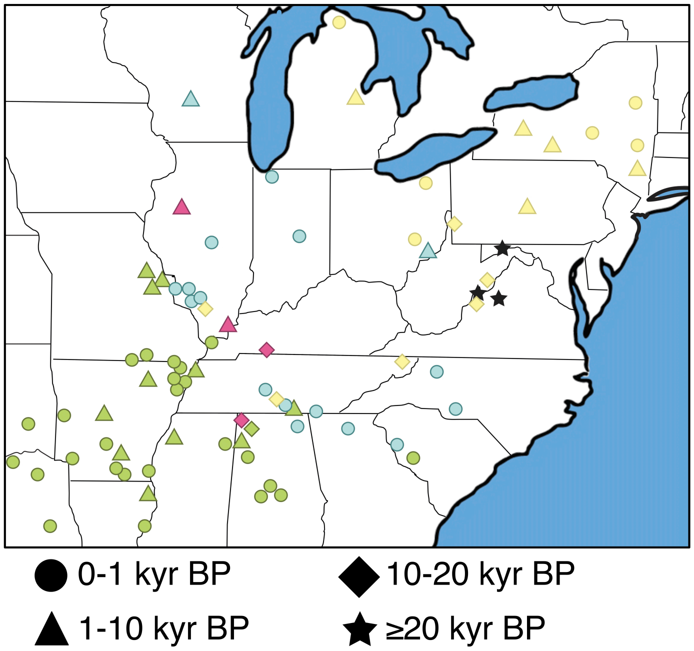
In order to understand the impact of passenger pigeon ecology upon eastern American forests we have 1) reconstructed the species' recent population history from 41 complete mitochondrial genomes, 2) assessed the percentage of seed crops consumable by passenger pigeons using modeled gape sizes, and 3) experimentally evaluated the species' digestive impact on seeds using the living band-tailed pigeon.

Figure 1 *(following page)* A) Changes in biome cover as a percentage of known passenger pigeon range over the past 21,000 years in relative with indications of major forest community changes (dashed lines). Biomes represented are mixed parkland (MXPA), cool mixed forest (CLMX), warm mixed forest (WMMX), and temperate deciduous forest (TDEC). Tree symbols indicate the dominant community before and after a major transition – multiple transitions occurred in the WMMX biome, which are not denoted by tree symbols. B) Paleontological and archeological sites for which passenger pigeon remains have been positively identified. The color of the site indicates the biome present for the earliest known record of passenger pigeons for each given site (some sites contain passenger pigeon remains in multiple strata over which time the local biome changed).

A. Changes of major biomes within the known range of the passenger pigeon



B. Passenger pigeon paleontological and archaeological sites by age and biome



Section 2.2 Methods

Section 2.2.1 Mitochondrial genomes analyses

DNA extractions were conducted at the McMaster Ancient DNA Centre, Hamilton, Ontario, Canada, and at UCSC Paleogenomics Lab's Ancient DNA Lab facilities. Multiple negatives were processed with ancient DNA sample sets; the Ancient DNA Lab facilities are located in a separate building from the Modern Molecular Lab facilities in accordance with standard ancient DNA protocols (Cooper and Poinar 2000).

DNA was extracted from 39 historic and archaeological passenger pigeon samples using tissue-specific protocols for bones and toepads: the protocols outlined in Rohland, Siedel, and Hofreiter (2010) and Dabney et al. (2013) were used to extract DNA from bone samples. DNA from the toe pads was extracted by digesting them in a proteinase K buffer modified from the Qiagen Blood and Tissue Kit consisting of 150uL Buffer ATL, 30uL Proteinase K solution, and 20uL of 1M dithiothreitol (DTT), similar to that used by Fulton et al. (2012), in a rotation incubator at 56°C for 48 hours. DNA was purified from extract solutions using either 1) the Qiagen DNeasy extraction protocol (Fulton et al. 2012), 2) Qiagen Nucleotide Removal Kit according to the manufacturers protocol, 3) a phenol:chloroform:isoamyl alcohol and chloroform based solution (Enk et al. 2011), or 4) "in-house" silica columns (Rohland, Siedel, and Hofreiter 2010) using an extraction to binding buffer ratio of 1:2 and 30uL silica beads. No significant differences in DNA yield between different protocols for toepads was observed;

while bones processed following Dabney et al. (2013) yielded higher amounts of DNA than those processed via Rholand, Seibel, and Hofreiter (2010).

PCR reactions were designed to confirm the presence of endogenous pigeon DNA. A 297bp fragment of the mitochondrial cytochrome b was targeted using the following primers: F2- (5'-CAAAGAAACCTGAAACACAGG) and R3- (5'-GGGTTGTTTGAGCCTGATTC) (Shapiro et al. 2002). Polymerase chain reactions (PCRs) using 1x HiFi buffer, 2.4 mM MgSO4, 20 µg rabbit serum albumin, 250 µM dNTPs, 400 µM of each primer, 1U Platinum HiFi Taq, and 1 µL DNA extract for a total volume of 25 µL were performed on a subset of samples. PCR reaction cycles consisted of an initial 12 minutes step at 94° C, proceeded by 50 cycles of 30 seconds at 94°C, 45 seconds at 48°C and 45 second at 68°C, with one additional step after all cycles of 1 minute at 68°C.

PCR reactions were cleaned using the MagNA bead protocol of Rohland and Reich (2012). The following molecular cloning, sequencing, and sequence analysis was performed using the methods outlined in Fulton et al. (2012). Sequencing was performed at the University of California, Berkeley (UCB) DNA sequencing facility.

Illumina sequencing libraries were constructed using the Meyer and Kircher (2010) protocol for all samples. Libraries were cleaned using Sera-Mag SPRI SpeedBeads (ThermoScientific) in 18% PEG-8000, to ensure that the small ancient DNA molecules would not be lost during the process.

Up to 1 million DNA reads were sequenced from the libraries using an Illumina MiSeq and v3 kit at the UCSC Paleogenomics Lab producing 75bp paired-end reads to evaluate the quality of the samples. For high coverage sequencing, 100bp paired-end read sequencing was outsourced to University of California, San Francisco Center for Advanced Technology and the University of California, Berkeley (UCB) DNA sequencing facility.

DNA reads were processed by removing Illumina adapter sequences and merging the paired reads using SeqPrep (https://github.com/jstjohn/SeqPrep), using a minimum overlap of 10 base-pairs between forward and reverse reads. Merged reads were mapped to the passenger pigeon reference mitogenome (Genbank KC489473.1) using MIA (github.com/udo-stenzel/mapping-iterative-assembler), which is an iterative short-read, reference based, assembler. Assemblies were visually inspected in Geneious (Kearse et al. 2012). Consensus was called at each position that had a minimum of three unique molecules mapped and created a final assembly for each individual mitochondrial genome. The resulting mitogenomes were aligned using MUSCLE (Edgar, 2004) and visually inspected the alignment using SeaView v.4.5.4 (Gouy et al, 2010).

To place the passenger pigeon mitogenomes in a phylogenetic context the a genealogical analysis was run using a coalescent-based method in BEAST v1.8.1 (Drummond et al. 2012). Bayesian skyline coalescent model was used, assuming a HKY+G nucleotide substitution model. Two different rates were used for modeling: 0.8% and 1% per million years, which are the slowest and fastest substitution rates of Columbiformes mitochondrial genomes according to (Nabholz, Glémin, and Galtier 2009). Two MCMC chains were run for 20 million iterations for each analysis, discarding the first 10% as burn-in. The MCMC chains were checked by eye using Tracer v1.6 (Rambaut et al. 2014) and calculated the maximum clade credibility tree using TreeAnnotator v1.8, (Rambaut and Drummond 2013) which was annotated using Figtree v1.4 (Rambaut 2009).

To visualize the relationships between mitochondrial genome haplotypes, a minimum spanning network (MSN) was estimated in Arlequin (v3.5) (Excoffier and Lischer 2010) using the Tamura-Nei substitution model. The proportion of differences between haplotypes was converted to number of differences through multiplication of the former by the length of the aligned passenger pigeon mitogenome (16,944 bp). Four summary statistics were selected to represent the genetic diversity of observed datasets: the number of segregating sites S, Tajima's D, Fu's FS and average pairwise distances π. These summary statistics were calculated with Arlsumstat v3.5.1.3 (Excoffier and Lischer 2010). A visual representation of the network was created using HapStar v0.7 (Teacher and Griffiths 2011).

Section 2.2.2 Passenger Pigeon Dietary Physical Constraints

Gape size is the limiting factor for the diet of species that must swallow their food whole (Wheelwright 1985). Bird gapes can be expanded owing to cranial kinesis (R. G. Bout and Zweers 2001), and in pigeons the mid-region of each rami of the lower mandible is flexible (Burton 1974). When considering the size of seed that a passenger pigeon was capable of eating, the extent of cranial kinesis and jaw flexibility can be estimated from band-tailed pigeons.

Measurements of the lower mandible at rest were taken from 10 band-tailed pigeons. Measurements of maximum flex at both the mid-jaw region (red bar, figures 3A and A5) and the jaw's articulation to the quadrates (blue bar, figures 3A, and A5) were then taken after cutting away the tongue, hyoid, and skin from the lower mandible leaving it articulated to the cranium. The ratio of expanded measurements to resting measurements was consistent between band-tailed pigeons (as well as between different columbid species, tables A7 and A8). The mean ratios of band-tailed pigeon values were used to model a maximum sized lower mandibular gape for the passenger pigeon using the mean relevant cranial measurements of passenger pigeons (table A10). A minimum gape model was produced based upon the average acorn size consumed by band-tailed pigeons (Fry and Vaughn 1977) and an intermediate sized gape model was calculated as the midpoint between maximum and minimum. These gape models (figure 3A) were used to measure the amount of mast crops which were physically consumable by passenger pigeons.

Most seeds consumed by passenger pigeons are too small to pose any challenge when swallowing. Acorns and chestnuts are the largest seeds that passenger pigeons were observed to consume. Not only is there considerable variation between species, but also considerable variation for intra-species seed sizes (Aizen and Patterson 1990). Mast crop samples were obtained from northern red oak, *Quercus rubra* (6 sites, 935 acorns), black oak, *Q. velutina* (4 sites, 415 acorns), white oak, *Q. alba* (3 sites, 618 acorns), and the American chestnut, *Castanea dentata* (4 sites, 83 chestnuts), from various locations throughout the eastern United States over two seasons (table A11). The caps of acorns were removed, as passenger pigeons were observed to pry acorns from caps before consumption (M. Gibbs 1892). Seeds were passed through the gape models to identify the percentage of each mast crop samples consumable by each model gape. Confidence intervals and standard deviations for all sample means and *p* values for mean comparisons were calculated in Microsoft Excel 2015.

Section 2.2.3 Passenger Pigeon Digestive Physiology

To infer whether tree seeds passed digestion intact by passenger pigeons I tested the passage of various seed types through the digestive tract of the band-tailed pigeon. The band-tailed pigeon is among the closest living relatives to the passenger pigeon (K. P. Johnson et al. 2010; Fulton et al. 2012) and analogous in diet ecology: eating seed, mast, and fruits of the same genera/families of plants as the passenger pigeon within its range. Acorns represent the largest seeds each species eat in common. Acorns within the range of the band-tailed pigeon exhibit the same range of size variation as acorns of eastern American forests (Aizen and Patterson 1990). Morphometrically band-tailed pigeons exhibit minutely larger skull dimensions to passenger pigeons with variance in size between both species overlapping (tables A9 and A10). Not only is the band-tailed pigeon observed to eat similar seeds to that of the passenger pigeon, but also it is physically capable of eating the same diet as the passenger pigeon. It can therefore be assumed that the band-tailed pigeon is a suitable physical and physiological model for the extinct passenger pigeon.

Four captive bred/reared band-tailed pigeons (2 males, 2 females) were obtained from Exotic Wings International, managed by Sal Alvarez. Germination competent seeds were purchased from commercial providers (section A1.1.3) of the following species: northern red oak, white pine, beech, red maple, yellow birch, and American elm. These were chosen to encompass the diversity in seed size and taxa that were consumed by passenger pigeons. Acorns and pine seeds represent seed types also directly consumed in the wild by band-tailed pigeons. Acorns and pine seeds also represent the major dominant tree taxa from coniferous and deciduous biomes over the past 21,000 years (Williams). No fruit species were included in this study due to limited availability.

Seeds were handfed to the band-tailed pigeons housed in separate and identical box-aviaries over the course of 4 trials allowing at least 36 hours of digestion before guano was collected for analysis. To account for potential impacts of each individual bird upon seed digestion, or impacts of seed species on the digestion of other seed species, the seeds were fed to the subjects in two regiments: 2 birds (1 male, 1 female) were fed a combination of all seed types at once over 4 trials (tables A4 and A5), and 2 birds (1 male, 1 female) were fed a single seed type each trial in random orders (table A6). Seeds were unaltered except in the case of red maple seeds in which the wing portions were cut off for efficient hand feeding. This alteration did not affect the integrity of the seed coat encapsulating the embryo and food store. This work was approved by the UCSC Institutional Animal Use and Care Committee.

Health and the gut microbiome of each bird will impact digestion of seeds. The birds were observed over the course of 3 months prior to experimentation to establish acclimation to the experimental environment and verify healthy physical condition. Guano samples from each subject were analyzed microscopically (40X magnification) for common Columbiform parasites by veterinary standards (Greiner and Branson 1994), e.g. protozoans (*Trichomonas, Eimeria*) and nematodes (*Capillaria*); all samples were negative for presence of parasites. No symptoms of bacterial infection were observed. The experimental environment induced no excessive stress. During acclimation the birds were fed a base diet of ZuPreem® fruit blend pellets and a custom mixed grain/seed feed and provided water from the same source. For the experimental period a commercial seed mix composed strictly of small seeds, but providing similar nutrient content, was substituted for the custom seed (all seed mix contents listed in section A1.1.3). Identical diet and water should maintain a consistent gut microbiota for the four birds when separated. These birds were communally housed prior to this period of acclimatization, a period in which they shared water dishes, exchanging saliva and consuming common biofilms. To continue this valuable exchange of microbiota between individuals water dishes were randomly rearranged every two days between the 4 experimental box-aviaries. It is assumed in this study that each bird represents similar replicate physiological environment for seed digestion.

The mass of guano produced for six days outside of the experimental period was recorded to verify that the experimental diet did not inhibit natural feeding intake of the birds.

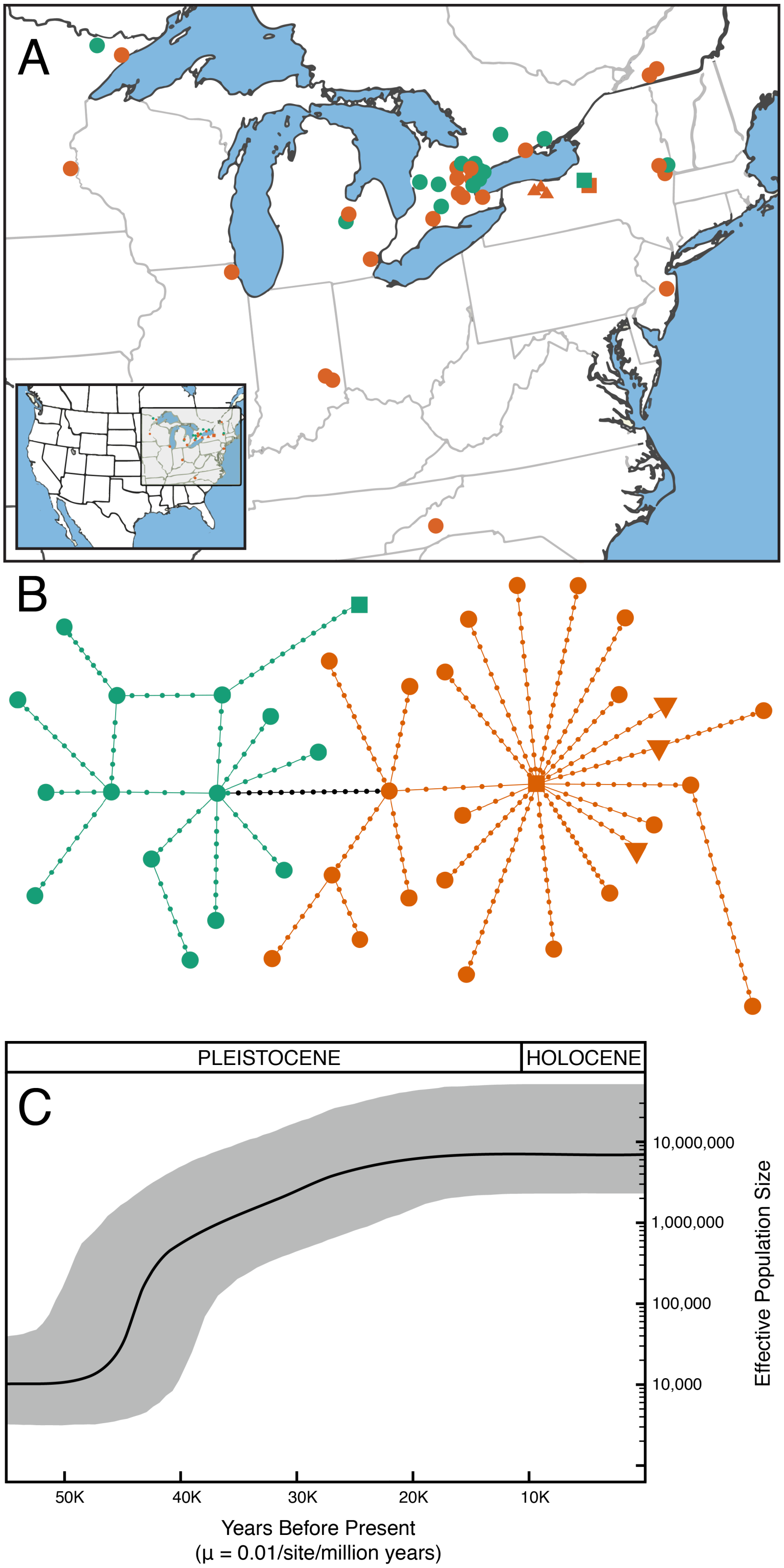
Section 2.3 Results

Section 2.3.1 Mitochondrial Population Genomics

The 41 mitochondrial genomes cluster into two major haplotypes (figure A5), both of which show a classic “starburst” topology (figure 2B), suggesting population expansion (Slatkin and Richard 1991). The geographic and temporal distributions of the two major mitochondrial haplotypes (figure 2A) indicate that passenger pigeon populations were not genetically structured, but existed as a single population. This is further supported by the recovery of both mitochondrial haplotypes from the same breeding populations—from individuals sampled at a single site on the same dates (table A1). The sample set contains a total of 255 segregating sites (S) and an average pairwise distance (π) of 23.06.

The topology of the mitochondrial tree, combined with the lack of geographic structure among mitochondrial lineages, suggests that the passenger pigeon population was expanding rather than declining during the late Pleistocene. This is further supported by negative estimates of Tajima’s D (-2.56), and Fu’s FS (-23.36). The timing and nature of population expansion can be inferred from the results of full-probabilistic reconstruction performed using the Bayesian coalescent inference package, BEAST 1.8.1 (Drummond et al. 2012), figure 2C. The results indicate an expansion in mitochondrial diversity, and thereby N_e, began around 45-55 thousand years ago for the slowest Columbiformes mutation rate, and 40-50 thousand years ago for the fastest rate. The population expansion continued until around 20,000-30,000 years ago reaching an effective population size around 7 million individuals.

Figure 2 *(following page)* A) Map of passenger pigeon tissue and bone samples localities and B) network of 41 mitochondrial genomes dating to the 1800s (circles), 1690s (squares), and ~4,000 yr BP (triangle). Orange represents haplotype 1 and blue haplotype 2. C) Bayesian skyline plot showing the population expansion of passenger pigeons according to the mitochondrial genomes alignment. Years are calibrated using the fastest mtDNA neutral mutation rate for columbiforms of 0.01 per site per million years.

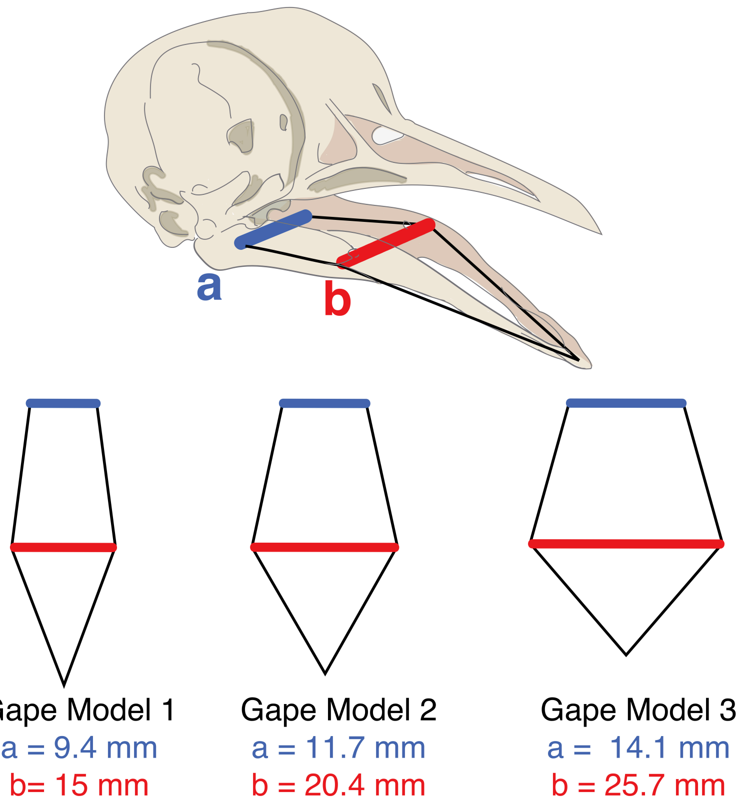

A
B
PLEISTOCENE
HOLOCENE
C
10,000,000
1,000,000
100,000
10,000
Effective Population Size
50K
40K
30K
20K
10K
Years Before Present
(μ = 0.01/site/million years)

Section 2.3.2 Consumable Seeds per Gape Model

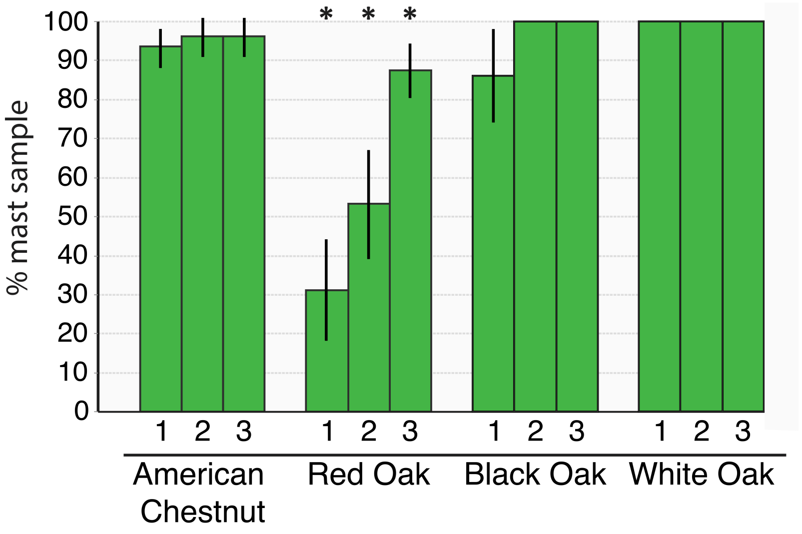
The mean percentages of seeds passing through each gape model are displayed in figure 2B. No statistical difference was observed between the percentages of mast crops consumable by all three gape models for American chestnuts (p= 0.1834 between gapes 1 and 2), Black oak (p = 0.1070 between gapes 1 and 2), and white oak; however for red oak a significant difference exists between each gape model (p = 0.0013 between gapes 1 and 2, p = 0.0009 between gapes 2 and 3). For white oak species (white oak and black oak), 100% of the mast crop is consumable by the minimum all gape models.

Figure 3 *(following page)* A) a diagram of a columbid skull showing the constant dimensions of the lower mandibular rami (black lines) and the expandable dimensions of the lower mandibular gape (blue line a, and red line b). The three gape models are shown to relative scale with actual measurements of expandable dimensions listed. B) Each green bar displays the mean percentage of mast crop samples passing through each gape model for each tree species – gape models indicated on x axis. 95% confidence intervals displayed and statistically different means indicated by asterisks above each bar.

A. Minimum to maximum predicted model gapes of an average passenger pigeon



B. Percentage of mast crops consumable by passenger pigeons based on model gape sizes.



Section 2.3.3 Gastrointestinal Seed Passage

No experimental seeds passed through digestion intact (Table 1). A total of seven acorns and a single beechnut were regurgitated during the study by three of the four pigeons. Six of the seven acorns were regurgitated from the crop intact within the first 12 hours after feeding, while a single acorn was regurgitated between 12 and 18 hours after feeding. This acorn was partially destroyed, likely having passed to the proventriculus where keratinous plates and gastroliths (swallowed stones) grind food content(Kaiser 2007). The acorn was too fragmented to germinate. This regurgitation likely reflects adjustment to the experimental diet, as these birds were not fed acorns prior to this study, rather than any natural proportion of regurgitation to passage. This is further supported by the observation that acorn regurgitations ceased after the second trial for birds continuously fed acorns (subjects 2 and 4), which suggests the first two trials were a period of acclimation to the experimental diet. Subject 3 did not regurgitate any seeds.

Table 1. Results of digestion after feeding seeds to band-tailed pigeons.

Seed type	Fed	Regurgitated	Passed digestive system intact
Oak Acorns	30	7	0/23
Beech	34	1	0/33
White pine	80	0	0/80
American Elm	60	0	0/60
Red Maple	45	0	0/45
Yellow birch	40	0	0/40

Dietary components macroscopically visible when dissecting guano included gastroliths (included in the base diet), keratin (from ingested feathers) partially digested seed material, and seed coat materials (figure A4). Seed coats from the base diet were observed rarely. Seed coat fragments from the experimental diet were restricted to acorns. Maple, elm, pine, and beech seed coatings were not observed.

No signs of indigestion (such as diarrheic guano) were observed during the experimental period. Hourly guano production in mass (grams/hour) during the experiment was not statistically different from guano production outside of the experimental period (p values for each subject between 0.1276 and 0.8718; table A3), indicating that food intake and digestion processing proceeded normally during the experiment.

Section 2.4 Discussion

Section 2.4.1 History of Abundance

The effective population size of the passenger pigeon over the past 21,000 years remains large and stable (figure 2C) through major changes in both the available habitat and community composition of the species former range (figure 1A). According to these results passenger pigeons were abundant during times in which conifer forests dominated its range when oak resources were extremely scarce and beech resources were virtually non-existent (Williams et al. 2004). In order to maintain abundance among changing forest habitats passenger pigeons would need to be superbly adaptable generalists. This indicates that past assumptions about dietary preference of passenger pigeons cannot be true. The seeming preference of passenger pigeons to consume acorns and beechnuts (Schorger 1955; Hung et al. 2014; Bucher 1992) is more likely due to the historic abundance of those mast crops rather than a behavioral or biological preference. From an ecological perspective the passenger pigeon appears to be a resilient species, contradicting previous hypotheses that its ecology predisposed the species towards extinction (Bucher 1992; Hung et al. 2014).

The past 21,000 years represents many lifespans/generations of the seed bearing tree species of the eastern United States forests: from 50-250 average non-overlapping lifespans or 525-5,250 non-overlapping generations of the dominant tree species known to be utilized by the pigeons for feeding, roosting, and nesting, (table A14). There is little doubt billions of passenger pigeons over hundreds/thousands of tree generations imposed selection on tree life histories and forest composition. Ecological interactions can drive selection in as little as one generation (Thompson 1998; Carroll et al. 2007). The life traits of trees shaped by passenger pigeons may have already begun to change in the absence of selection pressure. A reduction in seed sizes of Brazilian palms has been observed following the extinction of local avian frugivorous seed dispersers within a similar time frame to the absence of passenger pigeons in eastern North America (Galetti et al. 2013).

Section 2.4.2 Pigeons and Seed Crops

Past inferences of how forest composition was affected by passenger pigeons relied upon the assumption that all mast crops could be treated as equally likely to be consumed by passenger pigeons. The data clearly show that this is not the case for large seed bearing trees, particularly those of the red oak family (figure 3B, table

A11). Though this sample set is inadequate to extrapolate larger models of forest dynamics it presents the important point that the physical morphology of organisms has bearing on their niche space within the environment, a point often overlooked when considering ecological models for both living and extinct species. While a theoretical maximum lower mandible gape (the part of the gape in which every food item absolutely must pass in order to be swallowed) is presented, it is doubtful this maximum is biologically real. The minimum or intermediate gape sizes likely reflect the dietary constraints of the passenger pigeon more accurately. The reasoning is the manner in which pigeons must manipulate (grasp and station) food within the beak in order to swallow. For large small seeds pigeons use the tongue to move the seed back to swallow, but for large seeds the birds must "catch and throw" the seed until it is placed properly to swallow push through the lower mandibular gape (R. Bout and Zeigler 1994). Being able to station the seed properly is essential, because the ability of the lower gape to expand is passively induced by the forcing of the seed by the upper mandible, there are no muscles that can actively flex the lower mandible outward at the center of the rami (Burton 1974). In studies of how rock pigeons manipulate food with the beak and tongue to swallow it was found that as seed size increases the amount of time spent grasping, stationing, and swallowing the seed also increases. The maximum flexibility of the rami may simply not be achievable due to the extensive manipulation required, making the large seeds intractable.

The minimum gape size was modeled based upon a sample of acorns found to be consumed by band-tailed pigeons, which averaged 12.9 mm in diameter despite the average acorn of the mast crop measuring 17.2 mm. When a food crop is abundant in relation to the consumers, it is reasonable to assume that the birds would prefer smaller acorns, which require less time and effort to consume. The size of passenger pigeon flocks would not have afforded this leisure of preference, and likely would drive passenger pigeons to consume larger food no matter how cumbersome the task. It has been suggested that in ground feeding birds the time spent grasping and stationing seeds is minimized in order to maximize the time spent scanning for predators (R. G. Bout and Zweers 2001), which could explain why a flock of band-tailed pigeons would prefer smaller acorns to larger acorns; though, the dense social flocks of passenger pigeons likely precluded the need for each individual to maximize time spent scanning for predators. The possibility that the preferred size of acorns consumed by band-tailed pigeons is actually imposed more by physical limitations of food handling than behavioral imposition cannot be ignored. Also, given the band-tailed pigeon gape is slightly larger than that of the passenger pigeon, more weight towards the minimum gape size for passenger pigeons should be given, and the intermediate gape size considered to be a more accurate reflection of the extreme possible gape. From the data it would appear that passenger pigeons were only capable of consuming 31-53% of red oak mast crops compared to 100% of white oak mast crops, creating a very different selection pressure for each tree species.

Ellsworth (1985) was the first to attempt modeling how passenger pigeon disturbances shaped forests of the past. By assuming that passenger pigeons consumed red and white oak mast crops equally he deduced that the distributions of red and white oak could be attributed to passenger pigeon seed predation. White oak appeared to be most prevalent in areas in which passenger pigeons foraged during spring months. It was proposed that because white oak acorns germinate in the fall they were not available as a spring food crop among the mast uncovered by melting snow, which included red oak acorns. While autumn germination may have been selected for by passenger pigeon predation, it is unlikely to be the reason that red oak were not prevalent in spring foraging areas. Given that 47-69% of red oak mast crops are potentially not consumable by passenger pigeons would mean that red oak recruitment would be nearly as competitive in the same areas if passenger pigeon predation was the only factor affecting successful recruitment. In regards to the affect of passenger pigeons on the distribution of red and white oak it is more likely that differential response to passenger pigeon induced disturbances is the major selection factor over seed predation.

Given the species abundance, the impact of seed dispersal or predation would have large, but very different, outcomes for forest communities. The types of seed dispersal suggested by Webb (Webb 1986) include fecal, regurgitation, and crop contents of passenger pigeon carcasses. The results show that seeds regurgitated within 12 hours of consumption remained intact and are germination competent. There is no doubt that regurgitation has the potential for seed dispersal for wood pigeons. However, it is significant to note that regurgitation was restricted to large seeds and was not consistently observed. Regurgitation by passenger pigeons was historically observed, though any data regarding how often regurgitation occurred is lacking. Even seldom and sporadic regurgitation would result in considerable dispersal given population sizes numbering in the billions(Webb 1986) – however this does not provide the grounds for developing a mutualistic coevolutionary relationship as a seed disperser when confronting complicating trophic factors: intact regurgitated seeds could be secondarily consumed by other passenger pigeons or other bird and mammal species. Acorns are consumed by many species of birds and mammals in eastern North American forests.

The notion that passenger pigeons could be vectors of seed dispersal upon death certainly has the potential to have played a role in historic dispersal of seeds. Observations of the germination viability of crop contents of deceased eared doves, *Zenaida auriculata*, showed that only 35-50% of seeds were viable (Bucher and 2009); this level of viability was even observed for crop contents of carcasses that had decomposed for 30 days protected from scavengers. Secondarily 92% of eared dove carcasses left vulnerable to predators were disturbed to varying degrees, releasing crop contents. The seed contents of passenger pigeon crops may have had similar levels of viability, however other acorn eating animals, including living passenger pigeons, likely consumed these post-mortem dispersed seeds, especially given the intense competition that passenger pigeon flocks imposed in their abundance. Therefore this mode of seed dispersal is difficult to propose being prevalent enough to warrant as an ecologically significant passenger pigeon impact.

When passenger pigeons nested/roosted for extended periods of time they did not provide the types of movements beneficial to seed dispersal (Schupp 1993).

Passenger pigeon movements while foraging were instead consuming seeds from a large area and then depositing them (as guano) in a concentrated area. The majority of time for regurgitation would either be at the source of the seeds or at the nest/roost. At the source the seeds are not dispersed. At the nest/roost they would encounter heavy competition from other seeds. In order for passenger pigeons to provide beneficial seed dispersal, regurgitation and deaths would have to occur during foraging flights and long migrations between roosts within less than 12 hours of ingestion according to the limited data observed in this study (seed retention in the crop has typically been shown to be less than 4 hours for most fruit pigeons (McConkey, Meehan, and Drake 2004; Lambert 1989; Wotton, Clout, and Kelly 2008)). With the compounding necessities to facilitate seed dispersal, the proportion of seeds consumed by a flock of passenger pigeon (and thereby removed from potential recruitment) by far out weighs the proportion of seeds that were possibly dispersed.

The types of seed dispersal most associated with mutualistic coevolution are fecal dispersal (fruit pigeons: McConkey, Meehan, and Drake 2004; Lambert 1989)) and cache dispersal (squirrels and jays: W. Johnson and Webb 1989). Passenger pigeons were never observed to cache seeds for later use, and their nomadic movements do not support such behavior. The results presented here strongly indicate that seeds and nuts were not dispersed fecally.

The passage of fruits could not be tested with the band-tailed pigeon subjects due to lack of availability of suitable fruits (i.e. fruits from genera and species consumed by both passenger pigeons and band-tailed pigeons, such as serviceberry, Amelanchier alnifolia, juneberry, Amelanchier spicata, pokeberry, Phytolacca decandra, huckleberry, genus Gaylussacia, elderberry, genus Sambucus, wild blueberry, genus Vaccinium). Though frugivorous pigeons have been found to pass seeds intact(McConkey, Meehan, and Drake 2004) as well as destroy seeds(Lambert 1989), granivorous pigeons and doves have typically been associated with seed destruction (David E. Blockstein, Maxwell, and Fay 1987; Goodwin 1970). A similar study to ours fed fruits to rock pigeons, Columba livia, and did not recover any intact seeds(Sayle 1924). The results with rock pigeons, which are granivores, suggest that a diet that includes seeds and nuts stimulates overall digestion of all dietary intakes. This would potentially extend to the more general diets of wood pigeons, which consume large quantities of both fruits and seeds. However, this point may not be significant regarding the passenger pigeon's large-scale impact on forests, being that fruit bearing trees are not the dominant trees of eastern forests.

Passenger pigeon predation likely drove the selection pressures of masting species; masting itself is regarded as a predator satiation reproductive strategy (Janzen 1971). Other traits passenger pigeon predation may have driven are patterns of asynchronous masting, germination timing, seed size, and tannin content – all methods by which trees evolve to cope with seed predation (Janzen 1971; Loehle 1988). It has been assumed that the "niche-related hypothesis", under which large seeds can exploit more versatile environments for successful germination, explains the wider geographical ranges of large seed species. Passenger pigeon predation offers an alternative hypothesis: larger seed-bearing trees can simply evade passenger pigeon predation. The data suggests that passenger pigeon predation certainly would have driven selection for larger red oak acorns and chestnuts.

Section 2.4.3 The Ecosystem Engineer

Now, with the established knowledge of seed predation, dietary constraints, and long-term abundance we can begin to postulate with better clarity the ecological niche of the passenger pigeon with regard to its coevolution to eastern North American forest communities. The duration of long-term abundance extends back tens of thousands of years, over which time it can be assumed that large flocks of passenger pigeons were generating canopy and understory disturbances relatively consistently in the same fashion observed during the 16th-19th centuries. Over multiple generations of contemporary plant and animal species, disturbance regimes would be favored in the wake of passenger pigeon driven disturbance/regeneration cycles. Indeed when examining native species of eastern North America disturbance dependent communities are found to dominate the landscape for both plants and animals (Fuller and DeStefano 2003; Hunter et al. 2001; Marc D. Abrams and Orwig 1996; Rentch, Fajvan, and Hicks 2003; Brawn, Robinson, and Thompson 2001; Nowacki and Abrams 2015). Recent analyses of past and present tree species' distributions found that disturbances, such as fires, account for forest structure more so than climate, and to this day, despite disturbance suppression, have not yet reached climate equilibrium (Nowacki and Abrams 2014).

The biology of dominant tree species in eastern communities displays a multitude of disturbance dependent characteristics. Recruitment of pine and hemlock (Marc D. Abrams and Orwig 1996) is facilitated by canopy disturbance, while oak is entirely dependent on such disturbances for recruitment (Carter et al. 2015; Dey 2014; Hutchinson, Sutherland, and Yaussy 2005; Hutchinson et al. 2012; Rentch, Fajvan, and Hicks 2003). Oak regeneration is initiated by canopy disturbances, which tree ring chronologies have shown were consistently stable over time, averaging a canopy disturbance event every three years (Rentch, Fajvan, and Hicks 2003; Marc D. Abrams and Orwig 1996). Canopy disturbance spurs many benefits to oak beyond regeneration including increased mast yield (Healy 1997) and the ability to outcompete shade-tolerant tree species (Dey 2014). Recent experimentation with American chestnut, the most abundant contemporary tree species of the passenger pigeons' former range, shows similar dependence on canopy openings for seedling recruitment (Dalgleish et al. 2015; Belair, Saunders, and Bailey 2014). The one thing all studies find in common when examining eastern North American forests is an overwhelming continuity of understory and canopy disturbance/regeneration cycles throughout history.

Forest disturbances are not just beneficial to trees. Disturbances induce cascading ecological benefits to eastern American vertebrate biodiversity (King and Schlossberg 2014). Early stage post disturbance successional habitats have been found to support higher biodiversity and bioadundance of birds, reptiles, amphibians, and certain mammals (e.g. ungulates) than mature closed canopy forests (Brawn, Robinson, and Thompson 2001; Fisher and Wilkinson 2005; Cantrell et al. 2013; Hunter et al. 2001). For several bird species, reproductive success increases among post-disturbance successional habitats (C. H. Greenberg et al. 2014). As later successional stages develop towards closed canopy habitats the community changes (e.g. bat and mustelid abundance increases with later successional stages, Fisher & Wilkinson, 2005). A forest supporting multiple successional stage habitats supports a much higher level of biodiversity and bioadundance than a closed canopy. For example bat species prefer mature stands for roosting but young successional stands for feeding (Litvaitis 2001). The largest challenges facing forest conservation today is the balance of maintaining successional and mature forests, made more pressing by the decline of successional habitats and species (King and Schlossberg 2014).

Fire has been deemed the primary factor shaping pre-European settlement eastern North American forest communities. The dominance of mast-bearing trees has commonly been attributed to Amerindian silviculture, which involved periodic controlled burns (M. D. Abrams and Nowacki 2008). Initial colonial period to industrial period activities and the near extinction of the American chestnut allowed oaks to expand during the early 20th century; but, after reaching the limits of attainable growth in the 1930's regeneration ceased – a phenomenon associated with fire suppression and timber harvest practices that do not mimic natural disturbances (Dey 2014). The importance of fire in relation to oaks specifically is eroding in light of new research. An evaluation of the causes of shifts from oak to maple communities reveals a complex suite of contributing factors including drought dynamics and changing herbivore populations (McEwan, Dyer, and Pederson 2011). Recent analyses of fire management on oak regeneration have consistently shown that fires alone do not stimulate oak regeneration; fire must be coupled with canopy openings in order for oaks to establish, and in fact, canopy opening alone will foster oak regeneration while fire alone will not (Hutchinson, Sutherland, and Yaussy 2005; Hutchinson et al. 2012; Dey 2014; Carter et al. 2015). In these studies it has been shown that fires do not create canopy openings, but only clear the undergrowth. Not only are canopy openings important for recruitment of masting trees, but canopy openings, not fire, are associated with higher species richness in birds (C. H. Greenberg et al. 2014). Canopy disturbances from storms are too sporadic to foster such widespread disturbance regimes.

A consistent source of canopy and understory disturbances are needed to create the ecosystems that dominate eastern American forests; if fire and storms are inadequate, what about the pigeons? When tree ring data was analyzed for 150-350 year old oaks the median intervals between regeneration periods induced by disturbances was found to be 3yr for small disturbances and 16 years for large disturbances (Rentch, Fajvan, and Hicks 2003); it’s possible these consistent regeneration cycles were induced by passenger pigeon roosting, which was observed to occur 3-10 years apart for most roosting sites (Schorger 1955).

I propose that passenger pigeons were ecosystem engineers of eastern North American forests, being the major disturbance generators shaping the composition, biodiversity, and bioabundance of forest habitats. The role of passenger pigeons in forests fits the definition of ecosystem engineers put forth by Jones, Lawton, and Shachak (1994): “the most obvious ecological engineering is attributable to species with large per capita effects, living at high densities, over large areas for a long time, giving rise to structures that persist for millennia and which affect many resource flows”. Jones, Lawton, and Shachak consider “patches”, such as successional forest habitats created by fire or biological factors, to fall under the category of habitat structures. The long-term abundance and nomadic movements of passenger pigeons provide a consistent widespread source of beneficial forest disturbances. Passenger pigeons imposed intense ephemeral, periodic, and consistent, impacts on forest communities. The roosting/nesting birds produced gaps in the canopy by breaking tree limbs and even whole trees when overcrowding perches – affecting the flow of sunlight in the system. The guano generated by the birds produced the same level of understory clearing as fires while simultaneously altering nutrient composition of soils. This same guano deposition also represents a major transportation of nutrients between forest habitats. Nesting/roosting flocks produced *both* the *understory* and *canopy* disturbances needed for recruitment and regeneration of masting trees. The expansive nomadic movements of passenger pigeons maintained a mosaic landscape of successional habitat types (patch dynamics) fostering long-term high levels of biodiversity. The flocks certainly impacted short term local biodiversity; the large flocks imposed heavy ephemeral competition on food resources, likely suppressing populations of other mast and fruit eating species, thereby allowing some usually rare species to proliferate. And lastly, passenger pigeons undoubtedly drove the evolution of many tree species' life history traits through differential levels of seed predation demonstrated by this study. By driving the evolution of trees, passenger pigeons affected the ecological evolution of all tree dependent species within a community.

Section 2.4.4 Concluding remarks

Most literature on passenger pigeon natural history does not consider disturbance generation as the primary ecological role of the passenger pigeon. Overall, disturbance generation has been considered a byproduct of the species' abundance, and not part of the coevolution of the passenger pigeon with its ecosystem. Here I put forth that disturbance generation is the long-term primary ecological niche of the passenger pigeon, driven by coevolution with the tree species used for food, roosting, and nesting. In this view of the species' natural history, abundance is a driver as well as a selected product of this coevolution characterized by antagonistic seed predation and protagonist canopy/understory disturbances. The disturbance generation of passenger pigeons produced an environment that favored the continued abundance and disturbances of passenger pigeons, creating a sustaining interaction. The disturbance regime fostered by passenger pigeons was ideal for species that benefit from disturbance, giving rise to the species communities still present in most eastern North American forests today.

While further data should be collected to more accurately model the impacts of passenger pigeons on forest ecosystems, the importance of testing physiological and physical assumptions of species' ecologies has clearly been demonstrated, especially in the case of species for which unfounded assumptions are often cited as common knowledge, such as the passenger pigeon. Future interpretations of eastern American forest ecology (both present, historic, and paleoecology) should account for the effects of passenger pigeons. Such considerations may have implications for forestry management and the recovery of rare disturbance-dependent species. It is likely that if passenger pigeon disturbances cannot be reproduced at the scale of entire biomes that the disturbance regime native to eastern North America will gradually be replaced by less diverse old growth communities. Many disturbance dependent vertebrates will decline and some may become extinct in eastern North America (King and Schlossberg 2014).

Section 3. Significance

Section 3.1 Bearing on Previous Work and Future Directions

The relationship of passenger pigeon abundance to forest paleoecology strongly suggests that passenger pigeons were capable of thriving in many different forest habitat types, a point that contradicts the previous assumptions. The notion that passenger pigeons were dependent on key habitat types stems from Schorger's 1955 compendium of the species' natural history. One key element that Schorger created which has caused some potential confusion regarding passenger pigeon ecology is the designation of a "principle breeding range" within the nomadic range of the species (shown in dark blue in figure 4). The majority of historically recorded nestings occurred within the principle breeding range, but as can be seen in figure 4 many sites were recorded with both small and large colonies outside this breeding range, some of which are considerably distant (Montana, Manitoba, Northern Ontario, Oklahoma, Texas, Mississippi). Many of these sites consisted of very different forest composition than those within the principle breeding range. While the principle breeding range definitely supported large colonies during the breeding season, it is possible that the paucity of recorded nestings outside this range owes partially to the lower densities of human settlements historically. Throughout Euro-colonial and Amerindian history it's likely that the birds bred more commonly throughout a larger range as forest conditions and climates shifted. While no historic accounts exist for the birds breeding in Tennessee, an archaeological site near Oliver, Tennessee, yielded 1,181 passenger pigeon bones dating to ~800 A.D (Jackson 2005). This abundance of bones could be from a roost, but very well could be from nesting birds (J. Greenberg 2014). The population genomics data presented here indicate that passenger pigeons must have exploited a diversity of nesting habitats throughout their history in order to maintain abundance during times when forests were drastically different from the conditions present during European colonization.

This principle breeding range has been a source of bias when assessing the habitat needs of the passenger pigeon. It has served to aid in the support of dependence on oak and beech mast crops (Bucher 1992; Ellsworth and McComb 2003). Ecological Niche Models (ENMs) constructed by Hung et al. (2014) and Stanton (2014) both predict suitable breeding habitat for the passenger pigeon using historic breeding sites to train their models. In both published models there is a discrepancy between the range of training and testing sites of the models, which appears to bias the model results towards a narrow range of suitable breeding habitat.

This is exacerbated in Stanton's work by the reinforcement of several model variables by beech distribution, which relies on the assumption that passenger pigeons primarily depended on beech mast crops. Hung et al.'s model predicts that almost no suitable habitat existed during the last glacial maximum (21,000 yr BP). This clearly could not be the case given that the effective population size of the species was reaching its peak and becoming stable just before this time. The results of these models would likely change if the variables for suitable habitat were relaxed to accept a wider range of forest types and conditions.

Hung et al.'s proposed hypotheses rest heavily on the assumption that passenger pigeons were dependent upon oak resources to survive. They use trends in oak abundance to predict trends in passenger pigeon census population size over the past 20,000 years for which their nuclear population genomics were not informative. Upper and lower bounds of passenger pigeon carrying capacity were calculated based on the average mast crop yield of white oak species and red oak species. Not only does the work presented in this thesis show completely different effective population trends than Hung et al.'s predicted carrying capacity trends, but the inability of passenger pigeons to consume 100% of red oak mast crops means that the calculated red oak carrying capacity is too high (this would increase the difference between minimum to maximum carrying capacity greatly). Modeling the environmental carrying capacity of passenger pigeons is a complex endeavor; future work should strive to encompass all major food sources as well as the gape size constraints of the pigeons.

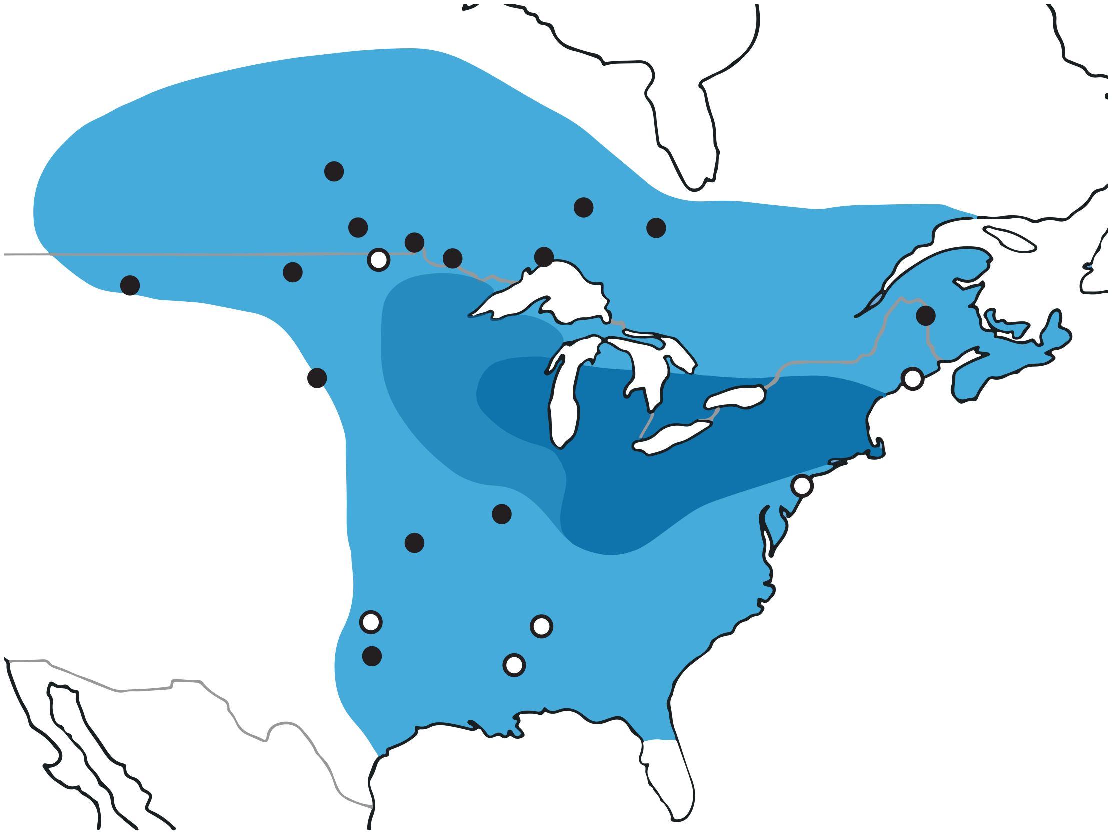


Figure 4. Map displaying the full nomadic range of the passenger pigeon (light blue) with Schorger's principle breeding range (dark blue). Nesting events recorded outside the principle breeding range are indicated by circles (small breeding colonies = black circles, large breeding colonies = white circles). The intermediate blue shaded range indicates an area in which many nesting records of both large and small colonies exist, but was omitted from Schorger's principle nesting range.

Census population estimates predating the 1880's are virtually impossible to calculate. The most famous attempts at calculating flock sizes are derived from estimating flock densities of migrating birds, which give estimates of flock ranging from 1-3 billion depending on the estimated flight speed and flock density (J. Greenberg 2014). An understanding of the population density required to cause canopy disturbances would not only provide insight into estimating the actual sizes of nesting and roosting colonies but also enlighten de-extinction efforts striving to target the number of birds needed to reproduce the beneficial effects of pigeon-generated forest disturbances. Passenger pigeons were recorded to deposit "several inches" of guano on the forest floor within roosting and nesting sites (Schorger 1955). The data collected on band-tailed pigeon guano production in this thesis can be used to make a rough population density estimate. The volume of dry guano produced by subjects 2 and 4 over a 7 day period was 100mL corresponding to an average of 28 grams (.28g/mL). The overall mean guano production per day was 2.4 grams per day, or 8.5mL. If a layer of guano 1 inch thick (2.54cm) was deposited over one hectare (2.54×10^8 mL) over the course of 30 days (8.47×10^6 mL/day) a flock of 985,785 band-tailed pigeons would be required to generate it: a density of ~99 birds per square meter. If this number is considered transferable to passenger pigeons, then the smallest recorded passenger pigeon roost (2.428 hectares, Schorger 1955) may have contained 2.4 million birds. One of the largest roosts recorded was reported to be 129.5 km^2, which by the same calculation would contain 12.8 billion pigeons, though this roost may have been exaggerated. Other reported large roosts were much smaller, such as 10.36 km^2, equaling ~1 billion pigeons, which appears to fall within most accepted estimates of flock size. These estimates are far from biological reality: they require that the pigeons could roost at a density of 99 pigeons per square meter over every square meter within a recorded roost, which would have been unlikely due to inconsistent canopy cover. Other problems with such methods include potential differences between the amount of guano produced by captive versus wild band-tailed pigeons and the assumption that guano production would be similar to that of passenger pigeons. Canopy disturbance offers another means to estimate population density: how many pigeons does it take to break tree limbs? Experiments testing the limb strength of various diameter branches of both young and old trees of many species will allow a means to estimate the number of passenger pigeons needed when perching to break branches (the recorded mass of an adult passenger pigeon was 255-340 grams (Schorger 1955). Data from such experiments could be combined with further guano collection studies of band-tailed pigeons to estimate the size of flock necessary to cause a large-scale canopy disturbance and the amount of guano that flock would produce over the disturbed area. This data could be used to design an experiment to directly test the keystone species hypothesis asserted in section 2.4.3.

The experiment needed to test the impact of passenger pigeon ecology on forests would be to simulate a passenger pigeon disturbance and compare the successional forest regeneration to other forest disturbances (i.e. fires, storms). Four experimental forest plots would be manipulated for the experiment:

Plot 1: controlled burn

Plot 2: canopy thinning

Plot 3: combination of fire and canopy thinning

Plot 4: passenger pigeon simulation

To simulate a passenger pigeon flock disturbance would involve scouring the understory to remove seeds and fruits (those consumable by passenger pigeons) over the course of the experimental period. A period mimicking a potential roosting, several weeks, can be used in order to allow other native species to consume food resources not removed by passenger pigeons. During the experimental period tree branches would need to be randomly broken and left upon the ground. The number of branches broken would be correlated to the modeled population size and density of the flock being simulated. In similar fashion the daily amount of deposited guano would be strewn about the plot. A large team of volunteers would be needed as well as a large source of pigeon guano – potentially available from squab farms. This process would mimic passenger pigeon dynamics as best as humanly possible.

All four plots should stem from the same forest community in order to compare differential responses of the forest community to each disturbance, yet each plot should be distant enough from each other to remove potential association effects between treatments. An ideal study would take place over a minimum of 4 years (a time frame used to assess success of American chestnut recruitment, Belair, Saunders, and Bailey 2014). This would allow 1 year to establish a baseline ecological community and 3 years to measure responses. Ideally wildlife and plant surveys would document responses in taxa of all trophic levels, not only paying attention to recruitment of major tree species. Changes in soil biochemistry should also be measured. Such an experiment would provide valuable data regarding strategies for forest management and assess the hypothesis that passenger pigeon disturbances produced similar beneficial effects to fire and storms.

Section 3.2 Significance to Conservation

Oak communities shifted to Maple dominated communities during the latter 20th century (McEwan, Dyer, and Pederson 2011). This trend is unlikely to change. The most effective forms of fostering oak regeneration combine the use of fire and canopy disturbance (McEwan, Dyer, and Pederson 2011; Hutchinson et al. 2012). Though tree dominance has changed and the living taxa represent a mere subset of those that once inhabited eastern North America (examples of other extinct and severely decimated taxa include bison, elk, wolves, mountain lions, Carolina parakeets, heath hens, greater prairie chickens, Bachman's warbler, peregrine falcon, burying beetle, bigleaf scurfpea, *Thismia americana*, American chestnut, cane bamboo), the common extant species still comprise disturbance dependent and disturbance adapted taxa – the one thing missing are frequent disturbances to maintain successional mosaics. If the proportion of successional forest habitats are not increased many of the extant species, in particular shrubland birds, may disappear entirely from eastern forests. Shrubland bird species have declined in eastern United States between 50-70% between Bird Conservation Regions since 1966 (King and Schlossberg 2014).

Maintaining forest disturbances is no simple challenge. It will take time to tell if fire and canopy thinning instrumented by humans will allow long term regeneration of oak communities and the recovery of declining disturbance species (McEwan, Dyer, and Pederson 2011). Managing disturbance regimes at the scale of whole forest biomes is infeasible, especially with the concerns of an increased human population throughout eastern forests and extensive woodlands owned as private property – 62% of all United States forests are privately owned by individuals, families, corporations, conservation agencies, First Nations tribes, and others ("Who Owns America's Forests? Forest Ownership Patterns and Family Forest Highlights from the National Woodland Owner Survey." 2008); many stakeholders are to be considered when dealing with forest management for biodiversity and productivity. Historically speaking, public opinion is usually opposed to destructive means of forest management (King and Schlossberg 2014).

The re-establishment of the passenger pigeon's keystone role in forest disturbance generation would offer a long-term solution to alleviate the burden of human facilitated disturbance management. The introduction of band-tailed pigeons to eastern states would replicate the dietary ecology of the passenger pigeon, but without some form of facilitated adaptation band-tailed pigeons will not assume the ecological role of generating forest disturbances, even if they were to become extremely abundant. The reason is that band-tailed pigeons are not colonial (Grinnell 1913). One historic report cites the presence of an aberrant colony of 14-17 breeding pairs of band-tailed pigeons in a single Douglas fir two years in a row (1933-34), but other accounts of "high" nesting density put one nest per every 3-4 acres at most (Neff 1947). In sharp contrast historic accounts span 24-317 passenger pigeon nests in single trees with 100 nests per tree considered usual, equaling thousands of nest per acre (Schorger 1955). Certain morphological traits lacking in band-tailed pigeons are likely tied to the sexual selection dynamic inherent to passenger pigeon colonial breeding, such as the passenger pigeon's graduated tail and sexual dichromatism – both traits found to facilitate female choice in social birds (Thomas 1993; Owens and Hartley 1998). Both of these traits are extremely rare among the 70 classically defined species of New World columbids (D. Gibbs, Barnes, and Cox 2001). Among the passenger pigeons close relatives the *Patagioenas* genus these traits are completely absent. Only in the distantly related subfamily *Zenaidini* are degrees of these traits found. In the *Zenaidini* 3 closely related species exhibit graduated tails. Females of 11 species display duller shades of male coloration. Only 5 species rival the extreme sexual dichromatism of the passenger pigeon. Colonial breeding was wholly unique to the passenger pigeon among North American columbids. The European wood pigeon, while also compatible in diet, likewise would not replace passenger pigeon ecology. Nesting densities of the European wood pigeon range from 1.5-5.6 nests per acre (Murton 1958). European woodpigeons also exhibit territorial behavior over nesting sites (Murton and Isaacson 1962). While small flocks and single pairs were often observed breeding, social affinity was so strong in the passenger pigeon that as the species became extremely rare lone individuals were observed flocking with mourning doves, and 1 individual was observed associated with urban rock pigeons (Schorger 1955). To adequately restore the ecological role of the passenger pigeon will require intense human efforts or the facilitated adaptation of extant wood pigeons, such as the plan outlined by Revive & Restore's "The Great Passenger Pigeon Comeback" project.

Appendix 1

Section A.1 Supplemental Methods

A.1.1 Forest Paleoecology & the Passenger Pigeon

To evaluate the environmental changes experienced by passenger pigeons (shown in figure 1A) forest paleoecology data from Williams et al (2004) was used. Williams et al presented changes in major forest biomes as well as changes in biotic communities within each respective biome for North America from palynological data spanning modern to 21,000 years before present. The study presents a series of maps showing changes in biome cover/distribution over North America in relation to changing coastlines and glaciation from the last glacial maximum to present. Though several Pleistocene and early Holocene fossils have been attributed to passenger pigeons in the western United states, it cannot be verified that passenger pigeons normally lived outside of their historically observed range. For my analyses I focused on habitat changes in the historically observed range delineated by Schorger (1955) shown in figure 4.

Taking the maps provided in Williams et al (2004) I blacked out all of North America outside of the passenger pigeons observed range. The biomes remaining are shown in figure A1. Using Adobe Photoshop CC 2014 the number of pixels for each biome was counted. Only biomes for which passenger pigeons are known from historical observations to use for both breeding and nomadic foraging that could also be supported by fossil data were included for evaluation. These included the mixed parkland, cool mixed forest, warm mixed forest, and temperate deciduous forest.

Excluded biomes remaining within passenger pigeon range are steppe, taiga, and conifer woodland. In order to produce figure 1 in the pixel values of each biome (including the amount of range covered by glaciation) were calculated in ratio to one another and plotted with smooth curves between available temporal data points. Major biotic transitions within each were taken from Williams et al (2014).

An examination of lifespans and generation times given for several dominant tree species within the passenger pigeon range are noted in table A14. This data is taken from Johnson & Webb (1989), Loehle (1988), and Webb (1986).

Fossil and bone assemblages (shown in figure 1B) containing passenger pigeon remains were taken from Greenberg (2014), Jackson (2005), and Neumann, (1985), and the paleontological collections of the Carnegie Museum of Natural History (catalog numbers 30827, 12984, 30833, 30831, 30836). For each site in which passenger pigeon bones have been reported the earliest record was identified and used to assess the biome of the local environment corresponding to the same time period. To do this a map of United States and Canadian province borders was overlaid on top of the biome maps provided by Williams et al (2004). A sample of this is shown in figure A2. Many time periods reported for bone assemblages are recorded by stratigraphic or cultural designations demanding individual literature searches for many sites to identify the approximate time in years before present that passenger pigeon remains were deposited. The following resources were used to compile the data presented in figure 1B: Parmalee and Klippel (1982); Parmalee (1967a); Parmalee (1967b); Churcher et al. (1989); Walker (1998); Semken, Graham, and Stafford (2010); Michels (1968); Guilday (1982); and “This Week In Pennsylvania Archaeology: Excavations at Sheep Rock Shelter (36Hu1)” (2015).

A.1.2 Digestive physiology

The base diet provided the band-tailed pigeons during the acclimation period was composed of a custom seed mix, ZuPreme® fruitblend pellets, and commercial grit formulated by Volkman Seed Factory, Ceres, CA. The seed mix contained trapper peas, *Pisum sativum*, popcorn, *Pea mays everta*, red milo, *Sorghum bicolor*, black sunflower seeds, *Helianthus annuus*, and wheat, *Triticum aestivum*. The experimental period base diet substituted the custom seed mix for commercial Kaytee® wild bird food, comprised of black sunflower seeds, milo, corn, and millet.

Guano produced during the experiment exhibited noted differences in color and consistency than guano produced prior and post experimentation. Subjects fed a diet of all seeds (birds 2 and 4) produced dense, dehydrated guano nearly black in color. This guano was densely rich in acorn seed coat fragments and was constant for these birds throughout the study. Post experimentation, subjects 2 and 4 defecated a slightly more green and hydrated guano typical of pre-experimentation. Subjects 1 and 3 produced this same type of guano when fed acorns. Subjects 1 and 3 both produced somewhat hydrated, pasty reddish guano when fed beechnuts. When fed pine seeds, elm seeds, or maple seeds subjects 1 and 3 produced guano similar to pre/post experimentation. The similarities in guano found between subjects when fed acorns versus beechnuts and other seeds suggests that differing seed composition is responsible for differences in guano type. Changes in gut microbiota induced by the experimental diets could also account for differences in guano types produced, assuming that all birds began the study with a similar gut microbiota and subsequently experienced the same types of gut microbe community changes. Given that subjects 1 and 3 were fed seeds in differing orders, and therefore should have experienced a differing sequence of microbiota community shifts which one would expect to affect future shifts differentially, it is more likely that seed biochemistry is responsible for the consistent pattern of guano types observed.

Guano collected for mass measurements and during the experimental diet period was dried before dissection. Mold formed on several fecal samples prior to dissection. An example of an experimental guano sample taken from band-tailed pigeon subject 2 after fed a diet of all seeds is shown prior to dissection in figure A4B. Some of the contents dissected from the guano are shown figure A4C. All guano samples yielded similar results of macroscopically identifiable matter.

Section A.2 Additional Figures

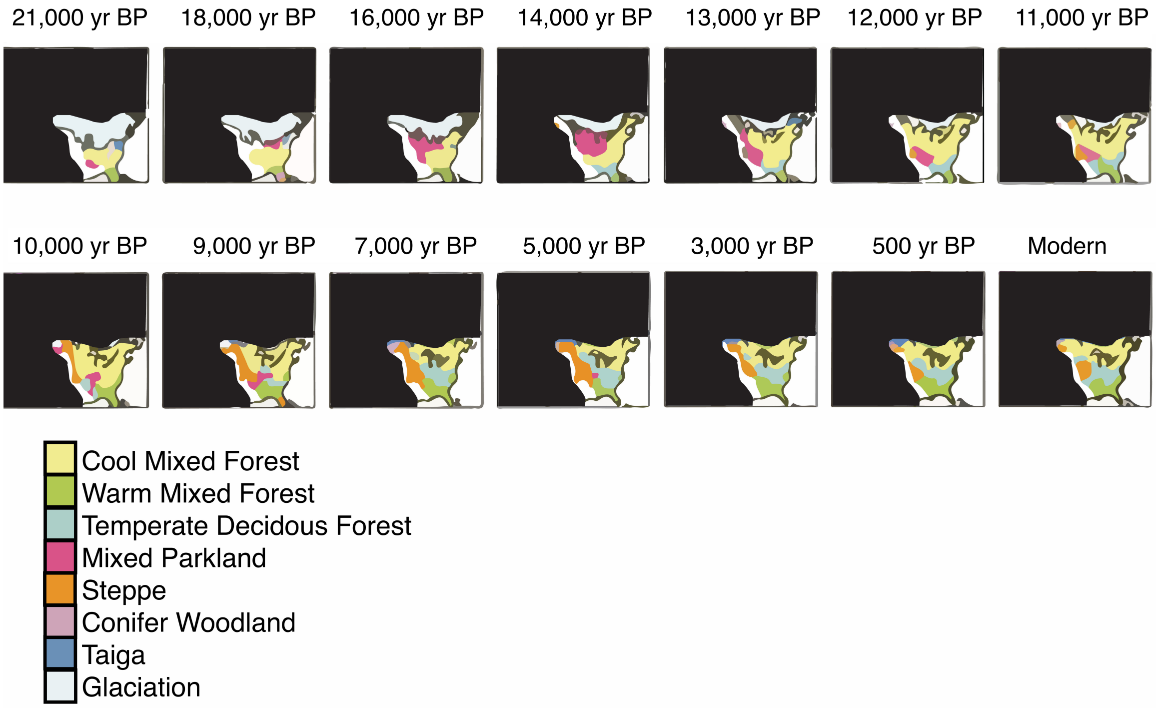


Figure A1. Forest biomes within passenger pigeon through time
Biome maps from Williams et al (2004) reformatted to count pixels in Adobe Photoshop with all areas outside of passenger pigeon range blacked out.

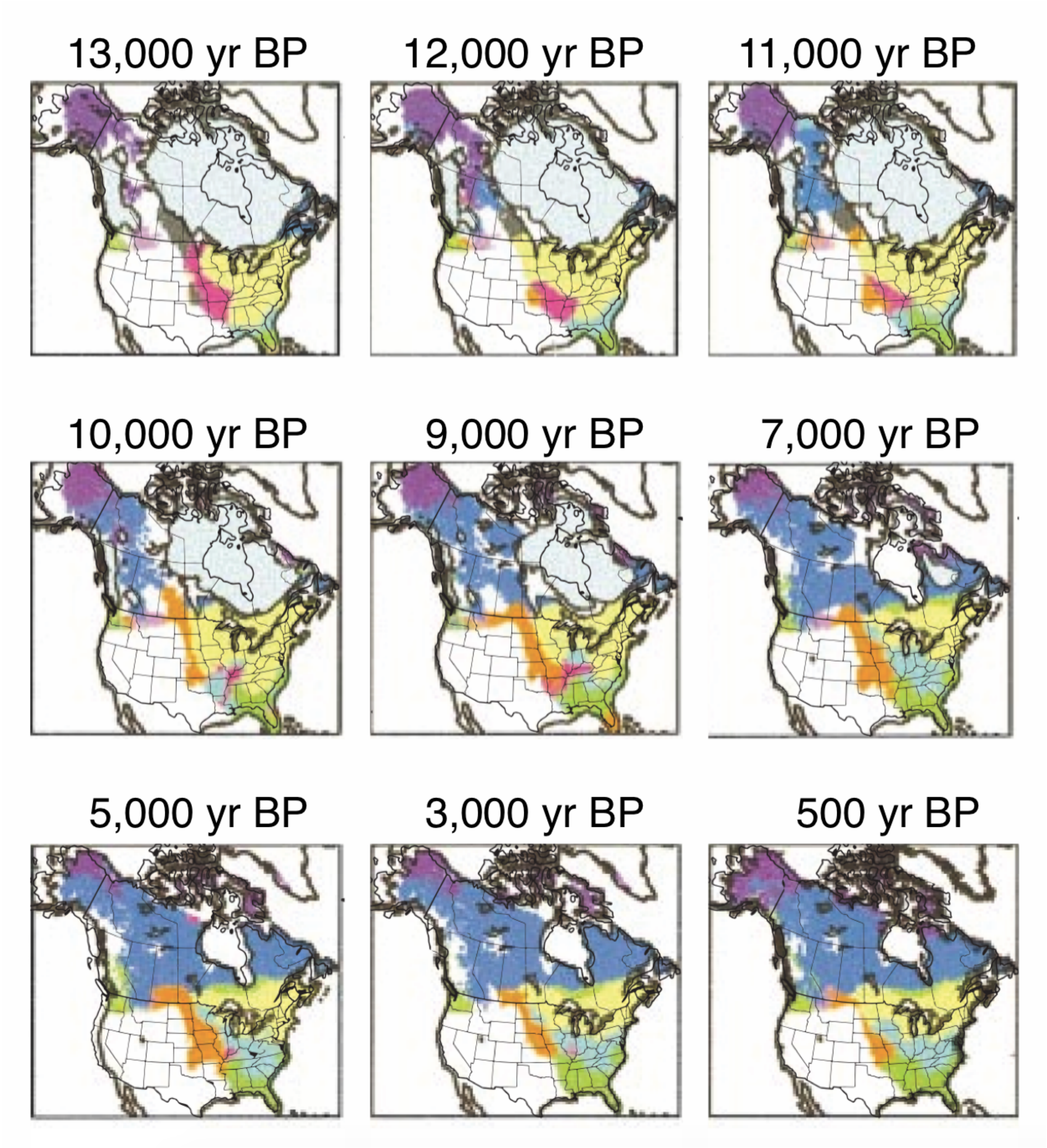


Figure A2. North American forest biomes through time, political borders Examples of biome maps from Williams et al (2014) with overlaid modern political borders. These maps were used to assess the biome present at known passenger pigeon fossil and bone assemblage sites for the age in which the oldest passenger pigeon remains were documented.

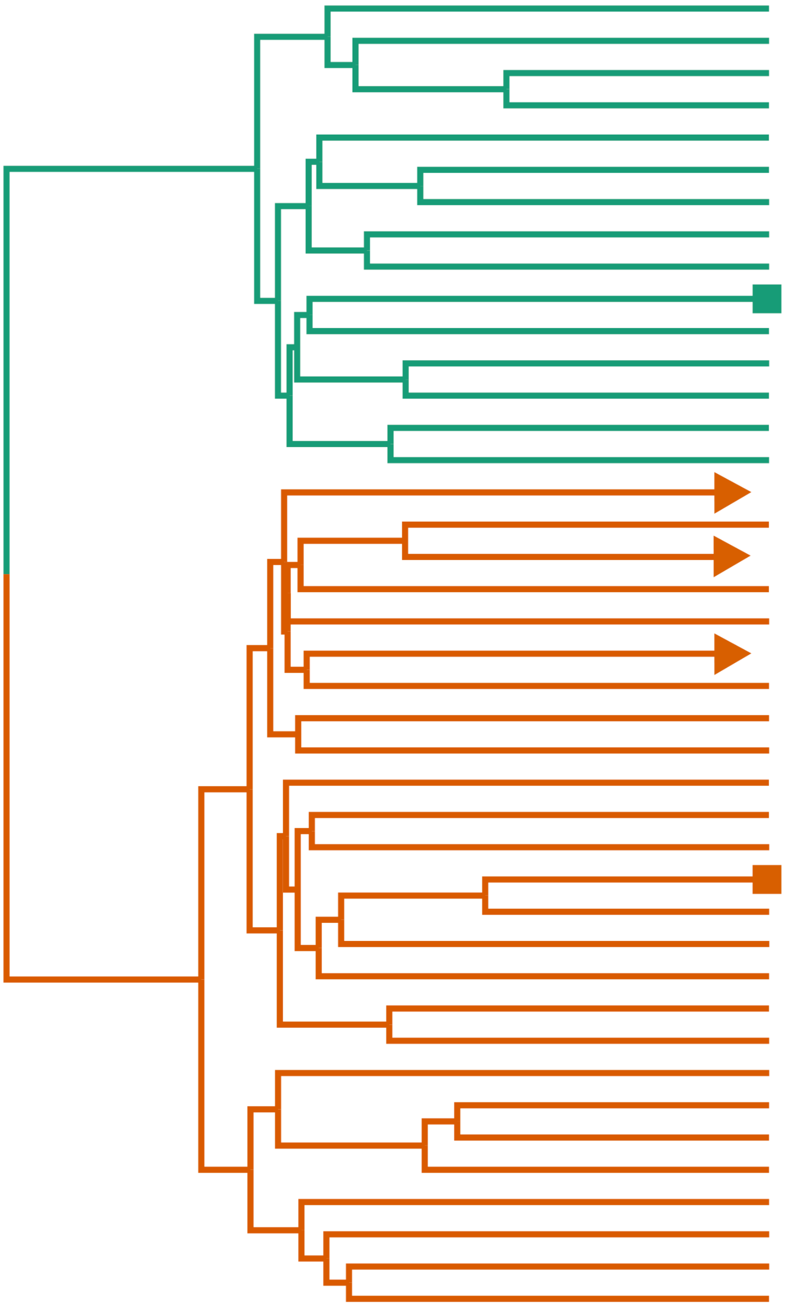


Figure A3. Phylogeny of 41 passenger pigeon mitochondrial genomes
Genomes from specimens dating to the 1690s are indicated by squares, those dating to ~4,000 years before present by triangles, and all other specimens date to the late 1800s. The two divergent haplotypes are colored to correspond throughout the document (figure 2, section 2).

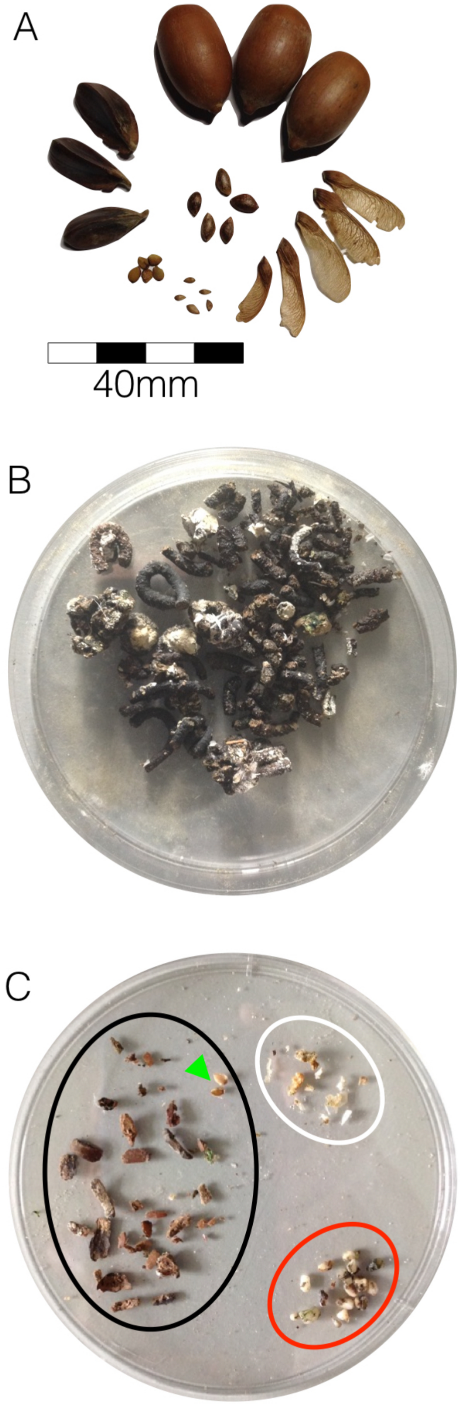


Figure A4. Experimental diet before and after digestion
A. Examples of seeds fed to band-tailed pigeon subjects (in configuration of the diet fed to subjects 2 and 4). In clockwise order from top to center: 3 red oak acorns, 5 red maple seeds, 5 yellow birch seeds, 5 American elm seed, 3 beechnuts, and 5 white pine seeds. B. Guano collected from subject 2, trial 1 before dissection. C. Examples of organic and inorganic guano contents: acorn and seed coatings circled in black, green arrow indicates two millet seed coats from the base non-experimental diet (commercial wild bird feed); digested seed material and partially digested feather material circled in white; pebbles from commercial feed circled in red.

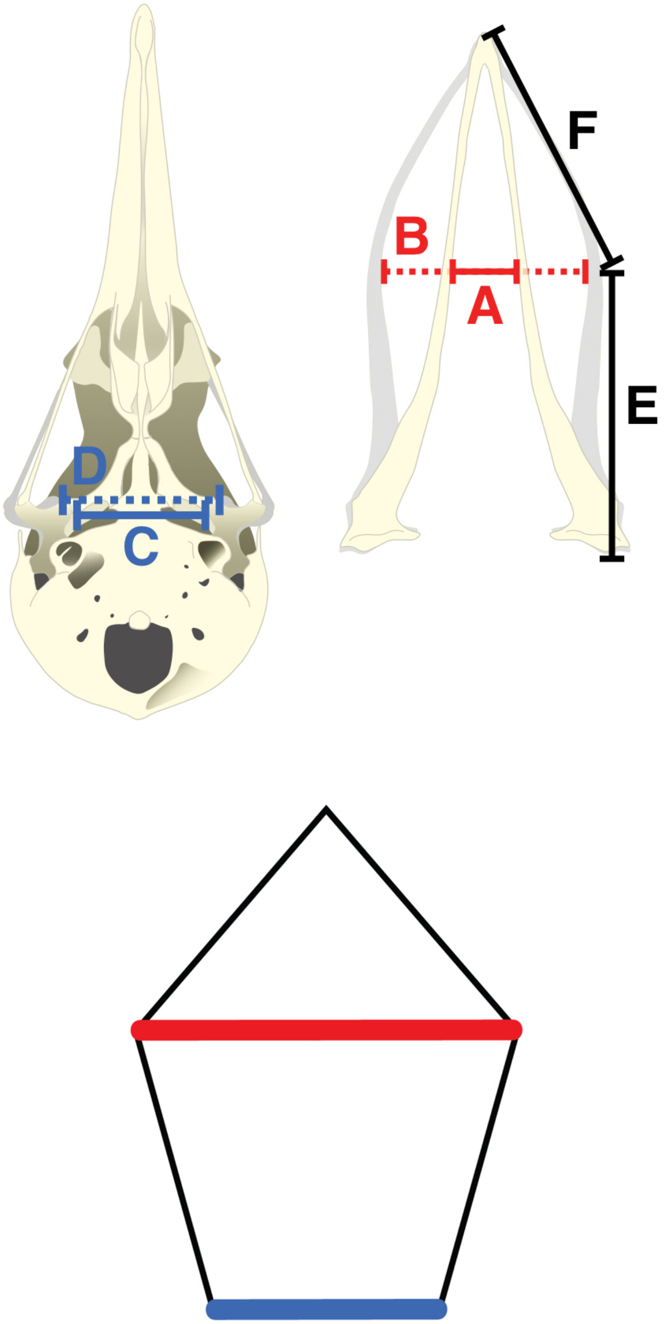


Figure A5. Columbiform gape model measurements
Diagram of the resting (A,C), expanded (B,D), and constant (E,F) measurements taken from columbid species' cadavers recorded in table A7.

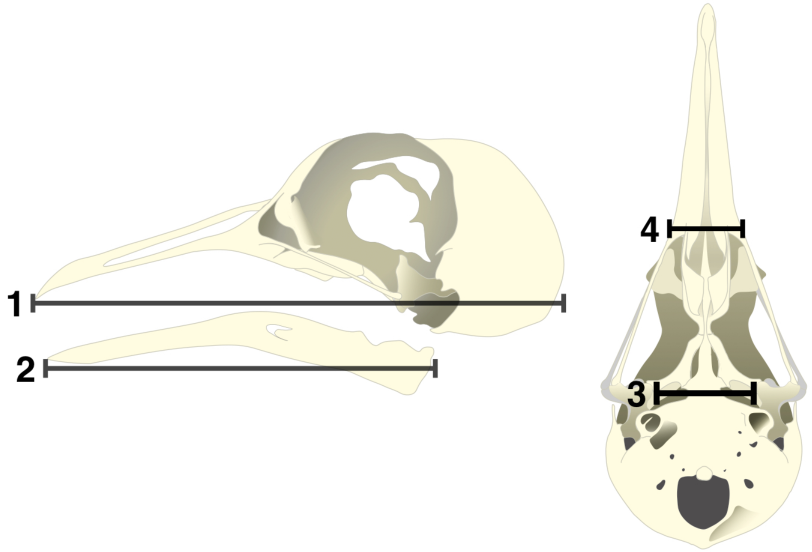


Figure A6. Columbiform skull morphometrics
Diagram of measurements taken from dry archival specimens of band-tailed pigeons and passenger pigeons recorded in table A9.

Section A.3 Additional Tables

Table A1. Sample set demography and associated mitochondrial statistics. Haplotype 1 is represented in blue and haplotype 2 by orange in figures 2 and A3.

Sample ID	Tissue	State, Country	Locality (City, County)	Sample Year	Average Coverage	Haplotype
FMNH 47395	toepad	New York, USA	Troy, Rensselaer	1860	239	2
FMNH 47396	toepad	New York, USA	Troy, Rensselaer	1860	135	2
FMNH 47397	toepad	New York, USA	Troy, Rensselaer	1860	238	1
ROM 40356	toepad	Michigan, USA	Wayne County	1898	385	2
ROM 33.6.203	toepad	Ontario, Canada	Peterborough County	1891	61	1
ROM 40359	toepad	Quebec, Canada	Mont-Royal Vale, Montreal	1888	45	2
ROM 67037	toepad	Ontario, Canada	Hamilton	1863	27	2
ROM 34.5.8.21	toepad	Indiana, USA	Oak Forest, Lake County	1888	15	2
ROM 34.5.17.1	toepad	Ontario, Canada	Embro, Oxford-Brant	1863	25	1
ROM 33501	toepad	Ontario, Canada	Lundy's Lane , Niagara RM	1860	10	2
ROM 34.3.23.2	toepad	Ontario, Canada	Toronto, Tannery Hollow	1871	692	1
ROM 80931	toepad	Ontario, Canada	E Dorchester, Middlesex	1885	26	2
ROM 40360	toepad	Quebec, Canada	Montreal	1880	62	2
ROM 30.10.17.1	toepad	Ontario, Canada	Perth County	1869	25	1
ROM 34.5.8.22	toepad	Indiana, USA	Oak Forest, Lake County	1888	61	2
ROM 34.1.5.2	toepad	Michigan, USA	Flint, Genesee County	1878	23	2
ROM 40363	toepad	North Carolina, USA	Weaverville, Buncombe	1894	21	2
ROM 40349	toepad	New Jersey, USA	Princeton, Mercer County	1875	81	2
ROM 73084	toepad	Ontario, Canada	Huron County	1875	21	1
ROM 34.1.5.1	toepad	Michigan, USA	Flint ,Genesee County	1878	45	1
ROM 76859	toepad	Ontario, Canada	Laurel, Dufferin County	1867	26	1
ROM 67104	toepad	Ontario, Canada	Rockwood, Wellington County	1875	21	2
ROM 34.6.7.1	toepad	Ontario, Canada	Bathurst St, Toronto	1880	38	1
ROM 91393	toepad	Ontario, Canada	Prince Edward County	1875	28	2
ROM 34.3.23.1	toepad	Ontario, Canada	Tannery Hollow, Toronto	1871	26	1
ROM 30.10.2.1	toepad	Ontario, Canada	Playter Crest, Toronto	1879	25	1
ROM 77384	toepad	Ontario, Canada	Dufferin County; Wellington	1872	28	2
ROM 34.4.19.1	toepad	Ontario, Canada	Don Valley, Toronto	1874	26	2
ROM 26535	toepad	Ontario, Canada	Toronto	1885	37	1
ROM 40355	toepad	Illinois, USA	Waukegan, Lake County	1892	20	2
ROM 40357	toepad	Ontario, Canada	Clarkson, Mississauga	1890	48	2
ROM 30.3.14.1	toepad	Ontario, Canada	Aldershot, Burlington	1890	40	2
ROM 36.4.27.1	toepad	Ontario, Canada	Cobourg, Northumberland	1886	48	1
DMNS 28.11078	toepad	Minnesota, USA	Minnesota northern border	1888	19	1
BN2-63	bone	New York, USA	Onondaga County	~1690	52	1
BN1-68	bone	New York, USA	Onondaga County	~1690	2.4	2
RMSC 3515	bone	New York, USA	Cole Gravel Pit, Livingston	~4,000 BP	20	2
RMSC 2794	bone	New York, USA	Cole Gravel Pit, Livingston	~4,000 BP	34	2
RMSC 3518	bone	New York, USA	Cole Gravel Pit, Livingston	~4,000 BP	48	2
BMNH749[1]	toepad	Minnesota, USA	Northern Minnesota	1879	31	2
BMNH1149[1]	toepad	Minnesota, USA	Southern Minnesota	1879	296	2

1. Specimens previously extracted and sequenced by Hung et al, 2014.

Table A2. Mass of guano collected from each band-tailed pigeon during and outside of the experimental diet period.

Collection Period	Day/Trial	Hours between collection	Guano collected (grams)			
			Subject 1	Subject 2	Subject 3	Subject 4
Non-experimental	1	24	2.30	1.70	2.18	1.70
	2	24	2.86	2.00	2.70	-
	3	24	4.54	1.92	1.61	0.82
	4	26	3.55	1.97	-	0.83
	5	24	1.60	1.90	-	1.52
	6	22	1.99	3.52	1.65	2.37
Experimental	1	36	4.48	2.85	2.63	1.73
	2	36	0.66	3.74	3.72	3.71
	3	36	4.50	8.60	2.72	5.85
	4	168	-	28.34	-	27.34

Table A3. Mean grams guano per hour and associated statistics.

Grams guano per hour				
	Subject 1	Subject 2	Subject 3	Subject 4
non-experimental period				
Mean	0.12	0.09	0.09	0.06
95% confidence interval	±0.03	±0.03	±0.02	0.03
Standard error	0.04	0.03	0.02	0.03
experimental period				
Mean	0.09	0.15	0.08	0.12
95% confidence interval	±0.07	±0.07	±0.02	±0.05
Standard error	0.06	0.07	0.02	0.06
Probability that non-experimental and experimental means fall into the same t-distribution (cut off 0.05)				
p value	0.5430	0.2188	0.8718	0.1276

Table A4 Band-tailed pigeon 2 digestion results

Seed Species	**# consumed**	**# passing GI tract**	**# regurgitated**
Trial 1 (36 hours)			
Acorn	3	0	1
Beech nut	3	0	0
Maple seed	5	0	0
Elm seed	5	0	0
Pine seed	5	0	0
Yellow birch seed	5	0	0
Trial 2 (36 hours)			
Acorn	3	0	1
Beech nut	3	0	0
Maple seed	5	0	0
Elm seed	5	0	0
Pine seed	5	0	0
Yellow birch seed	5	0	0
Trial 3 (36 hours)			
Acorn	3	0	0
Beech nut	3	0	0
Maple seed	5	0	0
Elm seed	5	0	0
Pine seed	5	0	0
Yellow birch seed	5	0	0
Trial 4 (168 hours)			
Acorn	3	0	0
Beech nut	3	0	0
Maple seed	5	0	0
Elm seed	5	0	0
Pine seed	5	0	0
Yellow birch seed	5	0	0

Table A5 Band-tailed pigeon subject 4 digestion results

Seed Species	# consumed	# passing GI tract	# regurgitated
Trial 1 (36 hours)			
Acorn	3	NA	3
Beech nut	3	0	0
Maple seed	5	0	0
Elm seed	5	0	0
Pine seed	5	0	0
Yellow birch seed	5	0	0
Trial 2 (36 hours)			
Acorn	3	0	0
Beech nut	3	0	0
Maple seed	5	0	0
Elm seed	5	0	0
Pine seed	5	0	0
Yellow birch seed	5	0	0
Trial 3 (36 hours)			
Acorn	3	0	0
Beech nut	3	0	0
Maple seed	5	0	0
Elm seed	5	0	0
Pine seed	5	0	0
Yellow birch seed	5	0	0
Trial 4 (168 hours)			
Acorn	3	0	0
Beech nut	3	0	1
Maple seed	5	0	0
Elm seed	5	0	0
Pine seed	5	0	0
Yellow birch seed	5	0	0

Table A6 Band-tailed pigeon subjects 1 and 3 digestion results

Seed species	Subject	Trial	# consumed	# passing GI tract	# regurgitated
Acorn	1	2 (36 hours)	3	0	2
	3	2 (36 hours)	3	0	0
Beech nut	1	1 (36 hours)	5	0	0
	3	4 (168 hours)	5	0	0
Pine seed	1	4 (168 hours)	20	0	0
	3	3 (36 hours)	20	0	0
Elm seed	1	3 (36 hours)	20	0	0
Maple seed	3	1 (36 hours)	5	0	0

*For each bird a single intact seed was found from the base diet – most likely a millet seed. These were not experimental seeds, and therefore are not considered in the results.

Table A7 Lower mandible kinetic measurements from recently deceased cadavers used to calculate model passenger pigeon gapes

Species	Institution[1]	Accession	Width of Mandibular Inflection Point (mm)			Width between quadrates (mm)			Length from inflection point to anterior and caudal tips of mandible (mm)		
			Resting (A)	Expanded (B)	ME[2] = B/A	Resting (C)	Expanded (D)	QE[3]=D/C	Caudal (E)	Anterior (F)	RAC[4]=E/F
Z. macroura	AAH	-	4.0	15.0	3.75	6.0	10.0	1.67	15.0	16.0	0.94
Z. macroura	CDFW	N14-223	4.0	11.0	2.75	7.0	9.0	1.29	13.0	15.0	0.87
Z. macroura	CDFW	N14-336	4.5	10.0	2.22	5.5	8.3	1.51	15.5	14.2	1.09
Z. macroura	CDFW	N14-335A	4.0	12.0	3.00	6.4	8.2	1.28	15.4	15.4	1.00
Z. macroura	CDFW	N14-335B	5.0	11.7	2.34	6.0	9.0	1.50	15.0	14.0	1.07
Z. macroura	CDFW	N14-424A	4.3	13.0	3.02	6.5	10.0	1.54	14.8	16.0	0.93
Z. macroura	CDFW	N14-424B	5.0	13.6	2.72	6.3	9.0	1.43	16.0	15.3	1.05
Z. macroura	CDFW	N14-424C	4.0	15.0	3.75	7.0	10.0	1.43	16.3	13.2	1.23
S. decaocto	CDFW	N14-423A	6.0	18.0	3.00	8.7	13.0	1.49	18.5	15.0	1.23
S. decaocto	CDFW	N14-423B	5.5	16.0	2.91	7.5	11.5	1.53	19.5	18.0	1.08
S. decaocto	CDFW	N14-423C	5.0	14.6	2.92	7.3	9.7	1.33	20.0	16.0	1.25
S. decaocto	CDFW	N14-423D	6.3	19.0	3.02	6.0	9.0	1.50	18.0	16.2	1.11
S. decaocto	CDFW	N14-423E	4.0	16.0	4.00	6.0	8.0	1.33	17.0	15.0	1.13
S. decaocto	CDFW	N14-423G	5.0	15.0	3.00	7.0	10.0	1.43	19.0	16.0	1.19
S. decaocto	CDFW	N14-423H	5.0	16.0	3.20	7.0	9.5	1.36	17.4	18.0	0.97
S. decaocto	CDFW	N14-423I	5.5	16.0	2.91	7.5	12.0	1.60	16.0	17.0	0.94
S. decaocto	CDFW	N15-191A	5.0	16.0	3.20	6.5	9.0	1.38	18.6	17.0	1.09
P. fasiata monilis	CDFW	N15-191B	7.0	18.0	2.57	8.0	12.4	1.55	25.5	20.0	1.27
P. fasiata monilis	CDFW	N15-191C	6.0	21.0	3.50	8.5	13.0	1.53	23.0	24.0	0.96
P. fasiata monilis	CDFW	N15-191D	8.0	24.0	3.00	8.0	12.0	1.50	20.0	22.0	0.91
P. fasiata monilis	CDFW	N15-191E	6.0	20.0	3.33	8.0	12.0	1.50	21.0	21.4	0.98
P. fasiata monilis	CDFW	N15-191F	5.5	22.0	4.00	9.0	16.0	1.78	22.0	23.0	0.96
P. fasiata monilis	CDFW	N15-191G	7.5	19.0	2.53	8.5	13.0	1.53	23.0	25.5	0.90
P. fasiata monilis	CDFW	N15-191H	6.5	19.5	3.00	9.0	13.5	1.50	21.0	24.0	0.88
P. fasiata monilis	CDFW	N15-191I	7.5	21.0	2.80	8.0	11.0	1.38	22.0	23.0	0.96
P. fasiata monilis	CDFW	N15-191J	7.0	18.0	2.57	8.0	11.0	1.38	22.0	21.0	1.05
P. fasiata monilis	CDFW	N15-191K	7.0	22.0	3.14	9.0	12.0	1.33	23.0	21.5	1.07

1. Alpine Animal Hospital (AAH), Palo Alto, CA; California Department of Fish and Wildlife (CDFW), Region 2 Wildlife Investigations Laboratory, Rancho Cordova, CA.
2. Mandibular expansion (ME)
3. Quadrate expansion (QE)
4. Ratio of anterior to caudal halves of mandible (RAC); anterior and caudal halves delineated by mandibular inflection point.

Table A8 Mean ratios of lower mandible kinetic measurements: expanding (ME, QE) and constant (RAC) parameters.

Species	**Mean ME ± 95% confidence interval (standard error)**	**Mean QE ± 95% confidence interval (standard error)**	**Mean RAC ± 95% confidence interval (standard error)**
Z. macroura	2.94 ± 0.40 (0.57)	1.45 ± 0.09 (0.13)	1.02 ± 0.08 (0.12)
S. decaocto	3.13 ± 0.23 (0.35)	1.44 ± 0.06 (0.10)	1.11 ± 0.07 (0.11)
P. fasiata monilis	3.05 ± 0.29 (0.47)	1.50 ± 0.08 (0.12)	0.99 ± 0.07 (0.11)
Probability that *Z. macroura* and *S. decacoto* means fall into the same t-distribution (cut off 0.05			
p values	0.4454	0.7956	0.1209
Probability that *Z. macroura* and *P. f. monilis* means fall into the same t-distribution (cut off 0.05			
p values	0.6938	0.4955	0.6047
Probability that *S. decaocto* and *P.f. monilis* means fall into the same t-distribution (cut off 0.05			
p values	0.6646	0.2792	0.0324

Table A9 Morphometric measurements from dry skulls recorded from band-tailed and passenger pigeons

Species	Institution	Accession	Sex	Total Cranial Length (mm)	Total Mandible length (mm)	Width between Quadrates (mm)	Width of cranium at nasofrontal hinge (mm)
P. f. monilis	MVZ	7387	?	58.13	42.63	10.27	8.77
P. f. monilis	MVZ	7831	?	57.28	41.15	10.37	8.77
P. f. monilis	MVZ	54522	Female	55.15	39.94	10.75	9.40
P. f. monilis	MVZ	54523	Female	54.91	39.53	10.46	8.96
P. f. monilis	MVZ	54524	Female	57.07	41.45	11.07	8.64
P. f. monilis	MVZ	54526	Female	55.89	39.97	11.41	9.42
P. f. monilis	MVZ	54527	Female	56.76	41.24	10.51	9.02
P. f. monilis	MVZ	63083	Female	55.93	41.90	9.99	8.24
P. f. monilis	MVZ	7836	Female	55.80	39.39	10.22	8.50
P. f. monilis	MVZ	7835	Female	54.63	41.25	10.47	9.81
P. f. monilis	MVZ	54525	Male	60.17	44.32	11.34	9.77
P. f. monilis	MVZ	65175	Male	56.84	40.74	9.41	9.11
P. f. monilis	MVZ	7832	Male	58.45	41.59	10.90	10.02
P. f. monilis	MVZ	7834	Male	56.69	41.57	9.08	9.08
P. f. monilis	MVZ	78336	Male	56.57	41.30	8.70	8.70
E. migratorius	AMNH	512	Female	53.90	38.40	NA	9.70
E. migratorius	AMNH	625	Female	52.90	38.50	NA	8.60
E. migratorius	AMNH	626	Female	53.60	38.80	NA	9.70
E. migratorius	AMNH	1052	Female	51.00	38.30	NA	9.20
E. migratorius	AMNH	1100	?	50.30	38.00	NA	7.40
E. migratorius	AMNH	3316	?	52.20	36.20	NA	9.30
E. migratorius	MVZ	84315	?	48.00	38.93	NA	NA
E. migratorius	USNMNH (VCL)	18520	?	52.50	48.00	9.80	7.90
E. migratorius	USNMNH	406	?	55.95	42.30	9.30	7.00
E. migratorius	USNMNH	292904	?	52.74	39.90	9.10	7.10
E. migratorius	USNMNH	224320	Male	54.00	41.30	9.35	8.50

Table A10 Mean values of morphometric skull measurements (from Table A9).

Species	Mean Total Cranial Length ± 95% confidence interval (standard error)	Mean mandible length ± 95% confidence interval (standard error)	Mean width between quadrates ± 95% confidence interval (standard error)	Mean width of cranium at nasofrontal hinge ± 95% confidence interval (standard error)
P. f. monilis (female)	54.52 ± 2.51 (3.63)	40.58 ± 0.68 (0.98)	10.61 ± 0.32 (0.46)	9.00 ± 0.37 (0.53)
P. f. monilis (male)	57.74 ± 1.36 (1.56)	41.90 ± 1.22 (1.39)	10.29 ± 0.70 (0.80)	9.34 ± 0.48 (0.54)
Probability that female and male means fall into the same t-distribution (cut off 0.05)				
p value	0.0509	0.1097	0.4453	0.3010
P. f. monilis	56.68 ± 0.74 (1.45)	41.20 ± 0.63 (1.25)	10.46 ± 0.28 (0.56)	9.08 ± 0.26 (0.51)
E. migratorius	52.74 ± 1.38 (2.34)	38.97 ± 0.99 (1.67)	9.39 ± 0.29 (0.30)	8.44 ± 0.65 (1.04)
Probability that *P. f monilis* and *E. migratorius* means fall into the same t-distribution (cut off 0.05)				
p value	0.0002	0.0016	0.0005	0.0965

Table A11 Results of mast crop samples passing through model gapes

Species	Locality	Year Collected	Passed Gape 1	Passed Gape 2	Passed Gape 3	Total
Castanea dentata	Zoar, New York	2014	18	18	18	20
Castanea dentata	Moss Lake, New York	2014	21	22	22	22
Castanea dentata	Cadillac Wexford, Wisconsin	2014	22	22	22	22
Castanea dentata	Bockenhaver, Wisconsin	2014	17	18	18	19
Quercus rubra	Sutton, Massachusetts (site 1)	2014	50	85	156	185
Quercus rubra	Sutton, Massachusetts (site 1)	2015	102	132	207	268
Quercus rubra	Sutton, Massachusetts (site 2)	2015	206	294	343	343
Quercus rubra	Wickoff, New Jersey	2014	13	20	36	45
Quercus rubra	Scioto County, Ohio	2015	12	32	57	66
Quercus rubra	Lawrence County, Kentucky	2015	4	13	27	28
Quercus velutina	Wayne, Pennsylvania	2014	60	77	77	77
Quercus velutina	Sutton, Massachusetts (site 2)	2015	48	65	65	65
Quercus velutina	Sutton, Massachusetts (site 3)	2015	170	172	172	172
Quercus velutina	Scioto County, Ohio	2015	95	101	101	101
Quercus alba	Bernardsville, New Jersey	2014	63	63	63	63
Quercus alba	Scioto County, Ohio	2015	74	74	74	74
Quercus alba	Sutton, Massachusetts (site 2)	2015	481	481	481	481
Quercus palustris	Kennet Square, Pennsylvania	2014	38	38	38	38
Quercus prinus	Lawrence County, Kentucky	2015	44	74	90	92
Quercus coccinea	Scioto County, Ohio		3	12	15	15

Table A12 Mean percentages of mast crop sample sets passing through model gapes

Species	Mean % Passed Gape 1 ± 95% confidence interval (standard error)	Mean % Passed Gape 2 ± 95% confidence interval (standard error)	Mean % Passed Gape 3 ± 95% confidence interval (standard error)
Castanea dentata	94 ± 5 (5)	96 ± 5 (5)	96 ± 5 (5)
Quercus rubra	31 ± 13 (16)	53 ± 14 (17)	87 ± 7 (9)
Quercus velutina	86 ± 12 (12)	100 ± 0 (0)	100 ± 0 (0)
Quercus alba	100 ± 0 (0)	100 ± 0 (0)	100 ± 0 (0)

Table A13. T-test results between mean values of seeds passing through each gape model.

Species	Probability that Gape 1 and Gape 2 means fall into the same t-distribution (cut off 0.05)	Probability that Gape 2 and Gape 3 means fall into the same t-distribution (cut off 0.05)
Castanea dentata	0.1834	1.0000
Quercus rubra	0.0013	0.0009
Quercus velutina	0.1071	1.0000
Quercus alba	1.0000	1.0000

Table A14. Lifespans and generation times of common eastern North American masting tree species.

Common name	Species' name	Average lifespan	Age of first seed production	Number of lifespans (non-overlapping) over 21,000 years	Number of generations (non-overlapping) over 21,000 years
Red Maple	*Acer rubrum*	130	15*	162	1,400
Yellow birch	*Betula alleghaniensis*	150	-	140	-
American Chestnut	*Castanea dentata*	100	12	210	1,750
American Beech	*Fagus grandifolia*	300	40	70	525
Blue spruce	*Picea pungens*	150	4**	140	5,250
White pine	*Pinus strobus*	200	5	105	4,200
White Oak[1]	*Quercus alba*	300	20*	70	1,050
Scarlet oak[2]	*Quercus coccinea*	80	20*	263	1,050
Red Oak	*Quercus rubra*	200	10‡	105	2,100
Hemlock	*Tsuga canadensis*	450	15	47	1,400
American Elm	*Ulmus americana*	175	15*	120	1,400

1. Longest lived eastern American oak species.
2. Shortest lived eastern American oak species.
*Average for genus.
**Age for *Picea glauca*.
‡Minimum age of first seed production.

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