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PALEOECOLOGY OF HAWAIIAN GEESE: STABLE ISOTOPE ANALYSIS

By

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ABSTRACT

PALEOECOLOGY OF HAWAIIAN GEESE: STABLE ISOTOPE ANALYSIS

By

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An evolutionary radiation of Hawaiian geese has been closely linked to the Canada Goose (*Branta canadensis*); the descendent species include the extant Hawaiian Goose or Nene (*B. sandvicensis*), the extinct woods-walking goose (*B. hylobadistes*), and the extinct giant Hawaiian goose (*Branta* sp.). Stable carbon and nitrogen isotope measurements of bone collagen were used to interpret dietary changes and habitat niche divisions between the three species. The osteological samples analyzed came from habitats that varied in precipitation, vegetation, elevation, and soil characteristics. In addition to the geese, samples of other vertebrate taxa were used to provide a broad basis for dietary comparison. The preservation of the collagen within the samples, which ranged in age from pre-contact Holocene to modern, was found to be exceptional. This is the first study in which isotopic data were used to relate speciation to changes in dietary niches of closely related bird species, and it could serve as a model for elucidating similar insular evolutionary radiations.

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Introduction

The Canada Goose (*Branta sandvicensis*), a common and widespread species native to North America, is considered to be the sister taxon to three endemic taxa of Hawaiian geese. Related to this radiation, and associated with morphological changes from the ancestral to the descendant species, I examined dietary differences and habitat partitioning between Hawaiian species of *Branta.* The three focus species are (1) the extant Hawaiian Goose or Nene (*Branta sandvicensis*), (2) the extinct woods-walking goose (*B. hylobadistes*), and (3) the extinct giant Hawaiian goose (*Branta* sp.). These geese must have been important herbivores on the islands where they occurred, yet little is known about their dietary and habitat preferences.

The following questions were addressed by analyzing stable carbon and nitrogen isotopic ratios of extracted collagenous bone protein from the three focus species, and from other taxa for comparison. Samples were selected from different elevations, habitats, and isolated versus overlapping ranges (Tables 1 and 2).

I. What were the dietary preferences of the two extinct species?
II. Did habitat partitioning occur between taxa with overlapping ranges?
III. How do elevational and climatic differences affect resource partitioning within and between species?
IV. How do environmental parameters affect the isotopic composition of the samples?

V. Do the archeological and sub-fossil data shed light on conservation issues of the extant, but very rare, Nene?

A key feature of this research is that it concerns an interspecific comparison of closely related taxa from different ecological regimes. Significant aspects of this project include: this is the first isotopic study in which the effects of speciation and colonization are interpreted in terms of their relationship to dietary niches of closely related birds; this study will help elucidate the avian paleoecology of Hawaii and Maui before human contact, leading to a better understanding of the evolutionary history of the archipelago's avifauna; this research provides information concerning habitat partitioning in closely related organisms with overlapping ranges, which is important for understanding insular evolution and ecology; and, finally, information on the dietary preferences and ecology of ancient Nene populations may help in the development of conservation strategies for living Nene by supplying data related to appropriate dietary and habitat essentials for long-term viability.

Geology and Climatology of Hawaii

The Hawaiian islands are significant for, among other reasons, being the most isolated island chain in the world (Carson, 1981). The nearest continent is North America, 3,800 kilometers (2,400 miles) away. The chain of islands has been isolated to this degree from the time it began forming during the Paleocene (Carson and Clague, 1995). This isolation has led to the very high degree of endemism seen on the islands today and in the fossil record. It has been estimated that nearly 99% of the Hawaiian biota is endemic (Olson and James, 1982b).

The Hawaiian archipelago consists of a long chain of islands and seamounts which successively formed over a fixed hot spot underneath the moving Pacific Plate (Figure 1) (Carson and Clague, 1995; Wagner and Funk, 1995). The immersed seamounts at the northwest end began forming approximately 75 to 80 mya (Carson and Clague, 1995). A bend in the chain occurred 43 mya, indicating a change in direction of movement of the Pacific Plate. As the plate moved northwesterly, successively younger islands formed to the southwest (Wagner and Funk, 1995). There are currently eight topographically significant islands, the oldest being Kauai which emerged about five mya (Olson and James, 1982a), and the youngest, Hawaii, which began forming 0.5 mya.

The exact spatial relationships between the islands during the geologic past are uncertain. It is difficult to determine if connections existed between islands, due to unknown island areas and changing sea levels (Carson and Clague, 1995). It is known, however, that there was a four-island complex, Maui Nui, present during the Pleistocene (Figure 2) (Carson and Clague, 1995). The connection between the islands was achieved through lowering of sea level to expose previously submerged connections between the islands of Maui, Lanai, Molokai and Kahoolawi (Olson and James, 1982a; Carson and Clague 1995). Maui Nui began to reseparate 300,000-400,000 ya, and was completely split into four islands about 150,000 ya (Carson and Clague, 1995). It is certain that Maui and Hawaii have never been connected by a land bridge; the closest distance between them has been estimated as 13 kilometers (8 miles) (Carson and Clague, 1995; Wagner and Funk, 1995).

Hawaii, the most recently formed island, is still volcanically active. This island has the greatest relief of the eight main islands, perhaps simply because it has not been as subjected to erosion and subsidence (Carson and Clague, 1995). There are three active volcanoes on this island: Mauna Loa, Kilawa, and Hualalai (Wagner and Funk, 1995). Newly forming to the southeast of Hawaii is the Lo'ihi Seamount, which is still submerged, but which will soon (in geologic time) emerge as the youngest Hawaiian island (Wagner and Funk, 1995).

There have been a few slight changes in climate during the time period encompassed by the samples used in this study (up to approximately 2600 ya). Compared to the modern climatic situation, drier conditions occurred from 1250 to 700 ya, followed by wetter conditions from 700 to 50 ya (Allen, 1997). During the colonization and radiation of the terrestrial geese, the Wisconsinian Glacial Period from 10,000 to 70,000 BP produced cooler and drier conditions within the archipelago (Hotchkiss *et al.*, 2000). At the glacial maximum approximately 18,000 ya, the sea level was 100 meters lower (Allen, 1997).

The current climate in Hawaii is most strongly influenced by trade winds and steep topography (Ralph and Van Riper, 1985). The trade winds nearly constantly blow northeasterly. These winds are quite moist, and produce high amounts of rainfall on the windward side of the volcanic mountains of the islands.

At elevations above 2000 m and on the leeward side of the mountains, conditions are semi-arid except for rare patches (Olson and James, 1982b; Ralph and Van Riper, 1985). Kona storms, resulting from rare southerly winds, occur two to seven times per year; these storms provide almost all of the annual precipitation on the leeward slopes (Ralph and Van Riper, 1985)

Hawaiian Archeological History

Polynesians first arrived in the uninhabited Hawaiian Archipelago as early as 1800 B.P. (Paxinos, 1998). The Polynesians discovered that the surrounding waters were not productive enough to support them on marine resources alone, so they created extensive agricultural systems with crop plants introduced from their home islands (Ralph and Van Riper, 1985). They developed tiered fish ponds, crop cultivation in mesic areas, and slash-and-burn field cultivation in drier regions (Olson and James, 1982a). The lowlands of the main islands were stripped of their native forests, and some smaller islands were completely denuded. The Polynesians also intentionally introduced foreign animals to the Hawaiian archipelago, including the dog, pig, and domestic fowl. These animals have been highly destructive to island avian populations by eating or chasing ground-nesters, rooting in the soil and disturbing ground-dwellers, grazing extensively on grasses, and invading niche spaces (Ralph and Van Riper, 1985).

Not only did the Polynesians drastically changed the habitat, but they also intentionally hunted the endemic avifauna. A native, David Malo, explains the use of the Nene by his people, "In its moulting season, when it comes down from the mountains, is the time when the bird-catchers try to capture it in the uplands, the motive being to obtain the feathers, which are greatly valued for making Kahili (*ceremonial cape*). Its body is excellent eating." (Kear and Berger, 1980). The large terrestrial flightless birds, such as the geese, ibis and other waterfowl, would have been easy prey for the Polynesians.

The effect the Polynesians had on the Hawaiian Islands was extraordinary. The lowland forests are believed to have contained the most diverse assemblage of plants and animals on all the islands (Olson and James, 1984). This massive degree of native habitat loss had a catastrophic effect on the avian biota. To date, 35 species of extinct birds have been recorded, and, as of 1991, perhaps 22 more sub-fossil species remain to be described. This is particularly striking considering that there are only 55 non-fossil endemic birds on the Hawaiian islands (Olson and James, 1991).

The European discovery of the Hawaiian Islands dates to Captain James Cook's 1778 voyage. When the crew arrived they found the islands densely populated with natives; an estimated Polynesian population of a quarter million people inhabited the islands (Ralph and Van Riper, 1985). Early explorers described the islands as having treeless, agricultural lowlands, and the smallest islands were completely bare of trees.

Shortly after Europeans arrived on the Hawaiian islands, they extended the clearing of forests to higher elevations, both for wood harvesting and for grazing introduced livestock (Ralph and Van Riper, 1985). Nearly one hundred percent of the archipelago's lowlands (<1500 m) have been altered by humans; native vegetation can no longer be found in these regions (Athens, 1997). Europeans also introduced several animals which have been detrimental to the endemic avifauna: cats, mongoose, two species of rats, cattle, goats, sheep and insects such as a carnivorous ant, parasitic flies and wasps (Ralph and Van Riper, 1985). The Europeans also brought with them domesticated fowl and other domesticated birds, which carried devastating non-native diseases, including Avian Pox Virus (Ralph and Van Riper, 1985).

Documentation of the archipelago's endemic birds has spanned the last two centuries; during this time many rare species have likely disappeared before being recorded (Olson and James, 1991). Some birds may have been greatly reduced in numbers by the Polynesians and then finally eradicated by European occupation. For example, the Dark-rumped Petrel was once common on all of the main islands, but is now found only at high altitudes on Maui and Hawaii. This drastic decrease is due both to hunting and predation by Polynesian- and European-introduced mammals (Olson and James, 1984). Historically, most of the endemic birds have been restricted to the higher elevation wet forests. This, however, is an artifact because fossil sites in lowland areas have representatives of the montane species, demonstrating that these birds once had a much larger range (Olson and James, 1982a).

Insular Evolution

Endemic species, those that are entirely restricted to one particular area, are characteristic of isolated islands. Islands offer an environment very conducive to rapid evolution and radiation. Speciation results from biological hardship, presence of an isolating mechanism, and geographic isolation (Mueller-Dombois, 1981). The extreme geographic isolation of Hawaii has provided a screen to the number and type of colonizers that reached the islands; only long-distance dispersers were capable of colonization. The native fauna lacked all non-flying mammals, land reptiles, amphibians, and freshwater animals (Mueller-Dombois, 1981). Once a founding population arrived on an island, it then spread into the available habitat and perhaps became specialized (*i.e.* adaptation), thus separating the one original population into subpopulations (Mueller-Dombois, 1981).

Where did the island species arise? One possibility is that a waif population from a distant continent may have colonized the island by chance, and then either remained relatively unchanged or undergone evolution and sometimes diversification. If speciation did not occur, the species would retain the approximate morphology and genetic identity of the founding population (Carson, 1981). Alternatively, radiation and speciation may have occurred, in which a new species is produced that is younger than the island it inhabits (Carson and Clague, 1995). Dispersal theory states that the species may have evolved on an older, pre-existing island, and then moved to nearby islands. In this way, the species is older than the island it inhabits (Carson, 1981; Carson and Clague, 1995). Finally, speciation may occur within the island itself. There is little evidence for sympatric speciation, thus, for speciation to occur the population must be divided (Coyne and Price, 2000). Geographic isolation may divide a population, causing genetic separation, perhaps eventually producing a new species. Some examples of within-island geographic isolation mechanisms include multiple volcanic mountains, the windward versus leeward side of the island, and separation of land areas by lava flows forming "kipukas", which are sections of older vegetation surrounded by a new lava flow (Carson, 1981).

A common change exhibited on islands is the loss of dispersal ability (e.g., flightlessness). Flight is a costly investment for birds to produce and then maintain (Gill, 1995; Chatterjee, 1997). Thus, when predation pressures are removed, maintaining flight capabilities may not enhance fitness. The development of flightlessness is associated with multiple morphological changes, including development of smaller wing bones, reduced size and eventual disappearance of the sternal keel, reduction in muscles for flight, obtuse articulation angle between the scapula and coracoid, and an unossified area between the ilium and ischium (Gill, 1995; Chatterjee, 1997). Development of flightlessness has often been linked to paedomorphosis, or retention of juvenile characters in the adult (Livezey, 1993). The increase in size (gigantism) often associated with loss of flight, may result in non-morphological changes such as: increased longevity and thermodynamic efficiency, and a greater capacity fto avoid starvation (Livezey, 1993). These traits would have led to greater fitness in a predator-free environment.

One group of birds particularly known for their tendency to evolve flightlessness in insular environments are the rails (Rallidae) (Livezey, 1998). A number of characteristics of these birds may have facilitated flightlessness: broad habitat range, high dispersal abilities in some and sedentary, behaviorally flightless, habits in others, and an opportunistic omnivorous diet in many (Livezey, 1998; Trewick, 1997). Most of the extinct rallids were flightless, and a diversity of different species are found on different islands throughout the world (Livezey, 1998; Trewick, 1997). Most taxa of flightless rails are believed to be dervied from separate founding events by a volant species, demonstating that speciation can occur rapidly when flight capability is lost (Trewick, 1997).

The first comprehensive model to explain island ecology and evolution was presented in 1967 by R. H. MacArthur and E. O. Wilson. In their book, The Theory of Island Biogeography, they endeavored to empirically explain the unusual fauna and flora often found on islands. They developed equations based upon such variables as land area, degree of isolation, and habitat diversity. Multiple regression analyses demonstrated that island area, which is correlated to habitat diversity, accounted for most of the variation in species numbers. Basically, the greater the diversity of habitats (and, thus, more land area), the greater the number of species an area can support (MacArthur and Wilson, 1967). They also determined that there is an equilibrium between immigration and extinction, which leads to a "stable" level of diversity. They found that there seem to be limits to the number of individuals present on an island, such that if one species becomes more abundant, another must likewise decline in numbers (MacArthur and Wilson, 1967).

Hawaiian Terrestrial Geese

The evolution of the Nene, the giant Hawaiian goose and the woods-walking goose is thought to have originated with the Canada Goose (*Branta canadensis*) (Figure 3) (Paxinos, 1998). It has been suggested that a flock of large-bodied Canada Geese or a very close relation was "blown off course" while migrating, and then became established within the Hawaiian archipelago (H. James, personal communication). As these three species evolved, they developed novel methods of exploiting the environment for resources. Instead of continuing as water-dependent geese, they became terrestrial herbivores that did not require open water for feeding or nesting.

According to a molecular phylogenetic study by Paxinos (1998), the large Canada Goose is basal to the evolutionary radiation of geese that occurred in the Hawaiian archipelago. The modern Canada Goose feeds mainly upon grass seeds in the summer, and various available plant material, such as waste corn and acorns, in the winter (Hanson, 1965). Thus, the basal taxon occupied a "browser" dietary niche, consuming a variety of plant material versus "grazers" that consume entirely grasses. Until recently, all subspecies of Canada Geese migrated between summer and winter locales. Pair bonds formed in the wintering grounds, and breeding only occurred near water environments (Hanson, 1965).

The evolutionary changes evident in the focus species relative to Canada Geese represent adaptations to an insular environment. At colonization by a "proto-typical large Canada Goose" (Paxinos, 1998), there were no terrestrial predators present in the archipelago, thus allowing the geese to reduce predator avoidance mechanisms and behaviors. The archipelago also offered the geese a year-round stable environment with abundant resources. This allowed the cessation of migratory behavior. Migration, or dispersal tendencies, would have rendered the birds unfit. Thus, reduced flight capability and even complete loss of flight arose in this insular radiation of geese (Figure 4).

A convergent radiation occurred in dabbling ducks, leading to a group of very large flightless herbivores, the moa-nalos or "lost fowl" (Olson and James, 1991; James and Burney, 1997). In comparison to the geese, the moa-nalos had a more reduced pectoral apparatus, and showed an increase in the size of their pelvic elements and overall body (Olson and James, 1991). Moa-nalos are believed to have fed a leafy material (*i.e.* browsers), and may have been specialized for consuming ferns. The remains of moa-nalos have been found distributed on Oahu, Molokai, and Maui, and typically at low elevation scrubland and forested sites, but a few have been found in high elevation rainforest on Maui (James and Burney, 1997). Thus, the moa-nalos would have been present in the range of the Nene and Woods-walking Goose. All of these birds occupied an herbivorous niche, and, more specifically, most likely a browser dietary niche, but the extent to which these flightless geese and "ducks" competed with one another is uncertain.

Branta sandvicensis (Nene)

The Nene is the only terrestrial goose to survive human arrival and colonization of the Hawaiian Archipelago. Interestingly, it is also the only endemic terrestrial goose that retained full flight capabilities; its sister taxa were all flightless. Since European colonization of the archipelago in 1778, the Nene were known only to live at high elevations on Hawaii in a habitat of scrubby trees and sparse ground cover. However, fossil discoveries have shown that before human colonization of the archipelago, the Nene was distributed throughout the main islands and was not restricted to high elevations (Olson and James, 1991). The Polynesians, therefore, evidently caused the vast reduction in Nene population numbers and range.

The extant Nene is on the Federal Endangered Species list, and is the state bird of Hawaii. At the time of Cook's landing, an estimated population of 25,000 birds inhabited the islands. In 1949, when only thirty wild and thirteen captive Nene existed, a much-needed recovery program was initiated (Black *et al.*, 1994; Black, 1995). Up through 1993, over two thousand captive-bred Nene have been released into eight sites within Hawaii, Maui and Kauai. Their population dynamics have been closely studied in hopes of establishing a self-sustaining population. Researchers (Black, 1995; Rave, 1995) have identified two important factors needed to help ensure the Nene's success. First, high elevation habitat is not solely able to support Nene populations; food must be obtained from lower elevation sites for the geese to survive. Thus, more habitat needs to be conserved for Nene use. Second, inbreeding depression within populations must be carefully controlled by introducing geese from other lineages to keep genetic diversity at sustainable levels (Black, 1994; Rave, 1995).

The extant Nene lives mostly in dry, vegetated areas on fairly recent lava flows, but also colonizes lowland mesic grassland and forested areas. Nene avoid dense, mesic forests. During the 18th century, they were recorded migrating to lowlands during the spring/early summer to feed on young grasses, and then returning to the uplands to feed mostly on berries. The Nene shows a dietary preference for leaves, buds, flowers, grass seed and berries (Bowler, 1994). The molting season coincides with gosling growth, resulting in the entire family being temporarily flightless, and thus being much more susceptible to predation (Berger *et al.*, 1980).

Branta hylobadistes (Woods-walking Goose)

Remains of the woods-walking goose were discovered in montane forests of Maui from sites as young as 1,000 years old, and from Oahu (Olson and James, 1991). Its range overlapped that of the Nene in mesic forests on Maui. The wing anatomy of this bird suggests that it was most likely flightless, but that it may have been able to sustain short bursts of flight (Figure 4). The morphological features of this species that suggested loss or near-loss of flight were the reduced wing size, reduction in pectoral girdle, and increased body and leg size (Olson and James, 1991; Paxinos, 1998). In the present research project, woods-walking geese from Maui only were analyzed.

Branta sp. (Giant Hawaiian Goose)

The first remains of the giant Hawaiian goose, which have the distinction of being the first avian fossil remains found in Hawaii, were discovered on the western side of Hawaii in a lava tube estimated to be between 3,000 and 5,000 years old (Wetmore, 1943; Giffin, 1993; Olson and James, 1982b). The skeleton was very large and certainly flightless (for example, the wings are very reduced in comparison to body size), originally suggesting a relationship to the moa-nalos. The only remains discovered of this bird have been on Hawaii, suggesting that evolution of the flightless giant Hawaiian goose occurred only within this island. The giant Hawaiian goose overlapped in range with the Nene on this island. The original name given was *Geochen rhuax*, but osteological studies have established that it is a member of the true geese (*Branta*) (Olson and James, 1991). The goose's extinction was most likely caused by the Polynesians, as supported by bone radiocarbon dates as recent as 510 BP, demonstrating the birds' coincidence with the Polynesian colonizers (Paxinos, 1998).

Stable Isotopes

Stable isotopes have been used for many years in ecological studies of both modern and paleontological biotas (Harrigan *et al.*, 1989; Ambrose, 1991; Matheus, 1995; Ostrom *et al.*, 1993). The biologically important elements (C, N, H, O, S) have naturally varying isotopes which can be measured to aid in addressing ecological issues including nutrient cycling, dietary sources and preferences, paleoecology, trophic level, and information concerning climate and habitat (Peterson and Fry, 1987; Schwarcz, 1991; Koch *et al.*, 1994; Koch, 1998).

Isotope values are measured using a stable isotope ratio mass spectrometer. They are expressed as:

$$\delta X = ((R_{\text{sample}})/(R_{\text{standard}}) - 1) \times 1000$$

where δX is ^{13}C or ^{15}N of the sample, and R is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. Positive values indicate that more of the trace (or heavy) isotope is present relative to the standard, whereas negative values indicate less of the trace isotope in the sample. The standard reference materials used are PDB (PeeDee Belemnite) for carbon, and atmospheric air for nitrogen (Peterson and Fry, 1987).

Isotopes of the same element behave very similarly to one another; however, they react at different rates. Molecules containing the heavier or trace isotope tend to react at a slower rate due to greater stability of the molecule and higher dissociation energies (Ostrom, personal communication). The reduced rate causes the trace isotope to be discriminated against in reactions, and the lighter isotope to be concentrated in the product (Ostrom, personal communication). This isotopic discrimination, or fractionation, results in a change in the isotopic ratio between the product and the substrate of a reaction, or the organism and its food resource.

The isotopic composition of an organism's tissues strongly reflect that of their food resource. Thus, isotopic ratios are useful in ecological studies (Koch *et al.*, 1994; Harrigan *et al.*, 1989). The isotopic value of the animal's tissue represents food that has been assimilated, with the exact period of time represented depending upon the turnover rate of the particular tissue analyzed (Gould *et al.*, 1997).

Carbon

Stable carbon isotopes were first measured in plant tissues in the 1950s, where a difference was noted between the three photosynthesis pathways (Craig, 1954; Katzenberg, 1992). The Hatch-Slack pathway (for C_4 plants) is utilized predominantly by tropical, arid-adapted grasses. This pathway tends to discriminate less against the trace isotope, leading to a $\delta^{13}C$ range of -8 to -16‰ (Gröcke, 1997). The majority of modern grasses are C_4, but exceptions are found in habitats at high elevation and high latitude (MacFadden *et al.*, 1994).

The Calvin-Benson photosynthesis pathway (for C_3 plants) is utilized by temperate trees, shrubs and some grasses. This form of respiration results in a $\delta^{13}C$ range of -23 to -34‰ (Katzenberg, 1992; Gröcke, 1997). C_3 grasses predominate in cool and moist environments, and do not occur below approximately 2000 m (6500 ft.) elevation (MacFadden *et al.*, 1994) The third photosynthetic pathway, which is utilized by cacti and succulents, lends intermediate $\delta^{13}C$ values (Katzenberg, 1992). This pathway is often disregarded in ecological studies because it is used only by a select few plant groups that are not ingested by many animals (MacFadden *et al.*, 1994; Gröcke, 1997).

An herbivore will have a carbon isotope value reflective of the plants it ingests (DeNiro and Epstein, 1978; Hobson and Clark, 1992; Koch *et al.*, 1994). The $\delta^{13}C$ difference between C_3 and C_4 plants is substantial enough that their isotopic signatures may be recognized in consumers. Thus, carbon isotopes may be utilized to determine if an herbivore is primarily dependent upon C_3 plants or C_4 grasses (Magnusson *et al.*, 1999) An herbivore that consumes a broad mix of leaves, berries, herbs, shoots and roots is termed a "browser", and those that consume entirely grasses are "grazers". This difference in herbivore dietary preference is reflected in the isotope values; browsers will have depleted $\delta^{13}C$ values from consuming both C_3 and C_4 plants, and a grazer would have higher $\delta^{13}C$ from the C_4 grasses isotopic signature (Iacumin *et al.*, 1998; Koch *et al.*, 1994).

Fractionation of carbon isotopes occurs during biochemical cycling; the average trophic fractionation for carbon is ± 1.0‰, (DeNiro and Epstein, 1978; Koch *et al.*, 1994; Schoeninger and DeNiro, 1984; Jahren *et al.*, 1998; Gröcke and Bocherens, 1996; Ostrom *et al.*, 1993), and the fractionation between diet and animal collagen is averaged at 4‰ (DeNiro and Epstein, 1978; Ambrose and Norr, 1998; Koch, 1998).

In addition to indicating differences between C_3 and C_4 plants, carbon isotope values are useful in determining microhabitat conditions. They have been used to distinguish between animals living in closed-canopy forest versus open grassland habitats (Gröcke, 1997; Marra *et al.*, 1998). This isotopic difference, or "canopy-effect," results from depletion of ^{13}C in closed-canopy forests due to biomass degradation (CO_2 uptake), and a gradient in light levels causing altered plant respiration efficiencies (Koch *et al.*, 1994; Bocherens *et al.*, 1996; Martinelli *et al.*, 1998). These two factors result in increasingly depleted carbon isotope values closer to the forest floor (Martinelli *et al.*, 1998). Elevation may also have an influence on carbon isotope ratios when using samples from a mesic environment. Vitousek *et al.* (1990) measured the isotopic ratios of a native Hawaiian tree from a sequence of elevations (approximately 70 m up to 2500 m). The average $\delta^{13}C$ value of all their samples was –28 to –26‰, similar to C_3 plants from continental locales. An interesting correlation was found: the $\delta^{13}C$ values increased with elevation, by almost 4‰ between the lowest and highest sample sites (Vitousek *et al.*, 1990).

Nitrogen

Historically, $\delta^{15}N$ values have been principally used to determine trophic position (Schoeninger and DeNiro, 1984; Ostrom *et al.*, 1993; Iacumin *et al.*, 1998). A number of studies have shown that an average 3 to 4‰ increase is associated with each trophic level (DeNiro and Epstein, 1981; Peterson and Fry, 1987; Ambrose, 1991). The 3 to 4‰ nitrogen fractionation that occurs in animals is due to excretion of isotopically light urine, amino acid processing and synthesis which results in ^{15}N enrichment of the product of metabolism (*i.e.* animal tissue) (Peterson and Fry, 1987; Ambrose, 1991).

Nitrogen isotope ratios may be used to distinguish between some plant types at the base of the food web. Given that atmospheric nitrogen has a $\delta^{15}N$ value of 0‰, N-fixing plants tend to have a nitrogen isotope value near zero (Peterson and Fry, 1987). For non-nitrogen-fixing plants, the $\delta^{15}N$ value reflects the isotope value of the soil, which is usually enriched in ^{15}N (Peterson and Fry, 1987; Vitousek *et al.*, 1989; Högberg, 1997). The climatic variables of the habitat are recorded in the soil $\delta^{15}N$ value. High temperature, high pH, high salinity and aridity all result in higher $\delta^{15}N$ values. The type of soil may also affect the nitrogen isotope value, variables such as increased depth, higher clay content and matured soils all tend to lead to higher $\delta^{15}N$ values (Ambrose, 1991; Vitousek, 1989). Nitrogen isotopes may also be used to determine dietary reliance on terrestrial versus marine foods, because marine resources are enriched in ^{15}N in comparison to most terrestrial environments (DeNiro and Epstein, 1981; Schoeninger and DeNiro, 1984). Very hot and arid environments, however, also lead to enriched $\delta^{15}N$ values in animals due to physiological reactions during heat and water-stress (Ambrose, 1991). This factor could possibly complicate the differentiation between terrestrial and marine resources. The climate of the Hawaiian archipelago is not extremely hot or arid; thus, the animals used in this study would not likely have been under heat and water-stress.

The variation of nitrogen isotope values in Hawaiian soils has been investigated by Vitousek *et al.* (1989). They took foliage samples from volcanic sites on the island of Hawaii from elevational and primary successional gradients. Vitousek *et al.* (1989) found, uncommonly, that non-nitrogen-fixing plants had lower nitrogen isotope values than N-fixing plants. Nitrogen-fixing plants, as expected, had $\delta^{15}N$ values near zero, ranging from –2.0 to +0.6‰, with a mean of –0.8‰. The non-nitrogen fixers exhibited $\delta^{15}N$ values from –8.0 to –3.0‰ (mean –5.1‰). The low values for non-N-fixing plants was attributed to: 1) soil nitrogen depleted in ^{15}N, which was more pronounced in younger soils; 2) fractionation between soil and plant that further reduced the $\delta^{15}N$ values; and 3) negative $\delta^{15}N$ values of N in precipitation, often the only source of nitrogen to undeveloped soils. The latter mechanism, however, was ruled out by the Vitousek *et al.* (1989), because the nitrogen content within precipitation over Hawaii is vanishingly small (5 kg/ha/yr). The researchers found a direct relation between soil age and $\delta^{15}N$: as soil age increased, the nitrogen isotope ratio also increased (Vitousek *et al.*, 1989). Comparing a 197-year old site to one of 67,000 years, the $\delta^{15}N$ increased by over 5‰ (-2.0 to +3.6‰, respectively). This is an important consideration that will have to be taken into account when evaluating the environmental effect on the sample isotope values, especially when comparing samples originating from Maui versus Hawaii. It must be ensured that an isotopic difference between samples is not simply a reflection of soil age differences, but is truly reflective of the animal's diet.

Collagen

The bone protein collagen is commonly used as a substrate for isotopic analysis of modern organisms (Cormie and Schwarcz, 1996). Remnants of collagen (i.e. collagenous proteins) also exist within fossils, and are utilized for ancient dietary and paleoecological analyses (Katzenberg, 1992; Ostrom *et al.*, 1993; Koch, 1998). Collagen is the predominant bone protein, accounting for 85 to 90% of the organic matter present in bone (Katzenberg, 1992; Gröcke, 1997). The turnover of bone tissue is very slow, such that collagen represents the animal's average assimilated diet over a long period of time, *i.e.* at least half a year in avian species (Hobson and Clark, 1992; Koch *et al.*, 1994). Measurement of the enrichment in ^{13}C of an organism relative to its diet due to fractionation during amino acid processing has given the values of: 0 to +4.6‰ (Ambrose and Norr, 1998), +2.8 to +3.7‰ (DeNiro and Epstein, 1978), and +5‰

(Koch, 1998). In this study, the mean value of +4‰ will be used when determining the isotopic composition of the diet from the collagen value.

It is important to understand the quality of collagen preservation in a fossil if one intends to utilize it to address paleoecological issues. Indigenous collagen is bone protein material that has not been contaminated by outside sources, and is isotopically reflective of the diet and niche of the animal during its life. DeNiro (1985) established a criterion for assessing the indigeneity of collagen in fossil samples. He showed that collagen samples with a carbon to nitrogen ratio (C/N) between 2.9 and 3.6 are isotopically representative of protein from the living organism. If a sample is outside of this range, the carbon and nitrogen isotope values might not be representative of the dietary habits and environmental conditions of the animal during its life.

Yield of organic matter can serve as another indicator of indigeneity as shown by McNulty *et al.* (in press) who subjected modern bone to high levels of heat for various amounts of time and humidity. The time-series of samples was analyzed for percent yield of collagen, C/N, and carbon and nitrogen isotope values. The results demonstrated that geochemical integrity of the samples was preserved even with very low percent yields of bone organic matter (collagenous protein), suggesting a 5% yield as a conservative criterion. These results are significant in application to true fossil samples because they suggest that indigenous isotopic signals can survive in fossil material, and they provide a new criterion for assessing the reliability of a sample's representative isotopic signal.

Methodology

The modern samples were initially prepared by a Dermestid Beetle colony within the Michigan State University Museum to remove exterior muscle and connective tissue. Processing of all bone samples for geochemical analyses was as follows (Figure 5): the bone was first mechanically cleaned with a nylon toothbrush and e-pure water, then cut into small pieces with a key saw. The pieces were sonicated in 4°C 1N HCl for 5 minutes, rinsed with multiple washes of e-pure water, and then sonicated another 10 minutes in e-pure water. The samples were then dried overnight in an evaporatory oven at room temperature, and then powderized in a SPEX CertiPrep 6750 Freezer/Mill. For each sample, up to three grams of bone went through a pre-wash procedure of stirring for one hour in a sodium buffer solution (1N NaH_2PO_4, pH = 6-7) at 4°C to remove humic substances. Each sample was centrifuged three times (4000 rpm) and rinsed with e-pure to remove and clean the supernatant. The samples were next demineralized in 1N HCl for approximately eighteen hours at 4°C.

Separation of the collagenous pellet from the acid-soluble non-collagenous protein (NCP) was achieved by high-speed (12000-14000 rpm) centrifugation. Each sample fraction was dialyzed at 4°C for five days in 3500 molecular weight cut-off dialysis membrane against many changes of distilled water. The samples were freeze-dried prior to geochemical analyses. Bone protein yield was calculated on the dry weight of the bone pre- and post-extraction.

The collagenous and non-collagenous protein fractions were prepared for isotopic analysis using a modified Dumas combustion (Macko, 1981). Purified gases from the samples were obtained by cryogenic gas separation and subsequent isotopic measurements performed on a VG-PRISM stable isotope ratio mass spectrometer (MicroMass). Also, a Carlo-Erba elemental analyzer interfaced to the PRISM mass spectrometer was used to determine the carbon and nitrogen isotope ratios and the C/N values (Wong *et al.*, 1992).

Results & Discussion

Assessment of Isotopic Indigeneity

As mentioned earlier, indigenous collagen has a carbon to nitrogen ratio between 2.9 and 3.6. With the exception of two samples, the carbon to nitrogen ratios of the sub-fossils are within this range (Tables 3 and 4). This lends support for the use of the collagen isotope data which appears to provide an indigenous signal that can be used to accurately interpret the paleoecology. There are two outliers: the sub-fossil giant Hawaiian goose (*Branta* sp.) sample four (GG.08.B4), and the modern Short-eared Owl, or Pueo, *(Asio flammeus)* sample one (PU.01.B1), whose C/N are 3.8 and 4.7, respectively. To further evaluate the integrity of these samples, collagenous protein yield (percent by weight) was determined, and the $\delta^{13}C$ and $\delta^{15}N$ values of these samples were compared to other measured values for these taxa.

The collagenous protein yields extracted from well-preserved samples should be between five and twenty percent. In a study of artificial diagenesis, McNulty *et al.* (in press) demonstrated that the isotope values of heated samples (at 100°C) with protein yields equal to or greater than 5% did not differ from those of unheated, non-diagenetically altered bone. The upper limit, 20%, is the percentage of collagen present in modern bone tissue. The majority of the sample yields were less than 20% including the modern samples, but nearly all were greater than 5% (Figure 6). On average, the sub-fossil samples produced lower collagen yields than the archeological and modern samples, which was as expected considering that the bone protein had undergone a longer period of diagenesis. Two samples fell below 5%: giant Hawaiian geese samples 3 and 4. Sample 4 (GG.08.B4) also produced a carbon to nitrogen ratio outside of the accepted range. The collagenous protein yield and C/N for this sample suggest that it has undergone extensive diagenesis, and may not produce an isotopic signal representative of the indigenous value. This assumption may be further tested by comparing the isotopic results to those of other giant Hawaiian geese. The same modern Pueo sample (PU.1) with a C/N value outside of the accepted range, also had an organic matter yield greater than 20% (24.3%). The failure of this sample to satisfy these two criteria suggests that it will yield isotopic results that are not reflective of the owl when it was alive. However, because it is a modern sample, diagenesis of the organic material is most likely not the cause for suspected non-indigeneity. Relatively little is known regarding the history of this sample; it is uncertain how long the specimen was along the roadside, or under what conditions it lived. Contamination of the bone material post-mortem may have led to the unacceptable C/N value and protein yield.

Isotopic Results

Stable carbon and nitrogen isotopic values were measured to establish the paleoecology of three closely related *Branta* species. A time-series (sub-fossil, archeological, and modern) of *B. sandvicensis* (the Hawaiian Goose, or Nene) was evaluated to determine whether the species experienced a shift in dietary niche subsequent to human presence in the archipelago. In addition, isotope values of collagen from woods-walking geese (*Branta hylobadistes*) and giant Hawaiian geese (*Branta* sp.) were analyzed to ascertain if a novel means of resource partitioning was developed between species to reduce direct competition with their sister taxon, the Nene. This project also endeavored to address the effect of environmental variables on the organic isotopic composition. The $\delta^{13}C$ values of the extracted collagenous protein are graphed against $\delta^{15}N$ values. The isotopic makeup of the herbaceous diet may be determined by adjusting for fractionation by subtracting 4‰ for $\delta^{13}C$ (DeNiro and Epstein, 1978; Ambrose and Norr, 1998) and 3‰ for $\delta^{15}N$ (DeNiro and Epstein, 1981, Peterson and Fry, 1987).

The Hawaiian Goose or Nene (*Branta sandvicensis*)

Isotope data for the Hawaiian Goose is highly variable (Figure 7). The $\delta^{13}C$ values range from –25.8 to –14.3‰, and the $\delta^{15}N$ from 0.53 to 12.9‰. Such a broad span of values suggests that diet varied among individuals, and that they incorporated a wide array of plant material. This is consistent with the known habits of the extant Nene (Bowler, 1994). In historical times, these geese were known to migrate seasonally between high and low elevations. In addition, they have a variable diet consisting predominantly of grass seed, berries, leaves, and shoots (Kear and Berger, 1980). The Nene occupies a "browser" dietary niche, in contrast to grazers that predominantly consume C_4 grasses. Thus, browsers would be expected to demonstrate a broad range of isotopic values.

The carbon and nitrogen isotopic values suggest an herbivorous diet consisting of few, if any, C_4 grasses.

The isotope values show one noticeable outlier in this data set: the archeological Nene sample four (NA.4). This collagenous sample yielded a very high nitrogen isotope value (12.9). This sample's locale was near the coast and at a low elevation. Marine resources are known to cause an enrichment in $\delta^{15}N$ values, which may be reflected in this sample's results. The values obtained for C/N and protein percent yield indicate indigeneity of the sample's isotopic signal. This sample is the only Nene from a coastal environment; the others originate from open grassland or scrubby forested sites. This difference may be the sole reason for the enriched nitrogen isotope value. Thus, perhaps this sample should be treated as an outlier of the data set based upon the unusual $\delta^{15}N$ value and the habitat origin. A Q-test statistical analysis for outliers was performed on this sample (Dean and Dixon, 1951; Dixon, 1951). The results indicate that the isotopic data from sample NA.4 may be rejected with 90% statistical confidence based upon the $\delta^{15}N$ value. The elevated carbon isotope value is most likely a result of a diet composed of a high percentage of C4 grasses (~90%). This sample is an outlier due to its unusual habitat, not from diagenetic alteration.

One other archeological Nene had a somewhat unusual nitrogen isotope value (NA.3, 0.53‰). The observation that the carbon isotope value of this sample falls within the average range of the other Nene supports the suggestion that the collagen retains an indigenous isotope signal. The carbon to nitrogen ratio and collagenous protein yield data also suggest indigeneity. The sample was from a very high elevation and dry site on Hawaii. The high elevation could have caused increased carbon isotope values, and aridity tends to lead to enriched $\delta^{15}N$ values, neither of which are reflected in the result. This suggests that the isotope values are reflective of the diet of this Nene rather than a result of climatic and geographic factors. Perhaps this particular individual was unusual in that it spent most of its time in forested, closed-canopy habitats consuming non-nitrogen-fixing plants on the forest floor. Both of these conditions would result in depleted carbon and nitrogen values, respectively.

The average carbon and nitrogen isotopic values for the sub-fossil Nenes are -18.73 ± 2.29‰ and 7.16 ± 1.64‰, respectively. Average isotope values for the archeological samples are lower than those of the sub-fossil data set, at –20.6 ± 1.75‰ and 4.49 ± 2.09‰ for $\delta^{13}C$ and $\delta^{15}N$. The modern samples average –21.77 ± 5.42‰ ($\delta^{13}C$) and 6.49 ± 0.37‰ ($\delta^{15}N$). The sub-fossil Nene have, on average, higher carbon and nitrogen isotopes than the archeological and modern samples (Figure 8). This result suggests that the Nene experienced a dietary shift over time. The sub-fossil Nene may have had a higher C_4 grass intake than the archeological geese (59.1% vs. 45.7%). This may be due to the sub-fossil geese being able to move between high elevation sites to low elevation grasslands. With the arrival of the Polynesians came extensive changes to the landscape, particularly in the low elevations. The changes may have ended the Nene's movements between elevations because the lowlands became covered with agricultural land and residential communities. The restriction in movement may have caused the shift in diet reflected in the isotopic values between the sub-fossil and archeological Nene. Decrease of the archeological Nene's range would have progressed as the Polynesians extended lowland habitat change over time and reduced population numbers through hunting and predation. The dietary change would not have been instant, hence the reduced fitness resulting from restriction of range to only high elevations would have also increased over time. Some populations of archeological Nene may have survived by finding hidden sites in which to feed and mate.

The data set was subjected to statistical analysis of variance (Campbell, 1974). The results conclude that the isotope average for the sub-fossil Nene is statistically different ($p < 0.05$) from the archeological average based upon the $\delta^{15}N$ value, supporting the conclusion that a dietary shift occurred between the time periods. The other comparisons, archeological and sub-fossil versus modern, are not statistically significant. These comparisons may have been obscured by the variability of the Nene diet, and low sample number. More samples of the extant bird, and more information concerning captive versus wild feeding, would help in addressing the question of dietary shift over time.

The modern comparison sub-set presents some interesting questions. For one, we see that the modern Nene $\delta^{13}C$ values encompass the two extremes of the sub-fossil and archeological Nene carbon isotope range. Although the sample size is small, some suggestion may be made as to what could lead to such variation. Since 1949 there has been an active conservation program to protect the dwindling population of wild Nene. The birds have been bred in captivity and many have been released into the wild. The modern Nene samples analyzed may have been captive-raised birds since two of the geese (NM.1 and NM.2) were banded, and it has been indicated that birds marked with leg bands usually originated in captive breeding programs (Stone and Pratt, 1994). It is uncertain how long they were in captivity and how long they lived in the wild before death.

One objective of this thesis was to assess how environmental factors may have influenced the isotopic values. To address this issue, we can compare samples that vary in elevation and/or precipitation. When this is done with the Nene-only data set, we do not see the expected pattern of enriched $\delta^{13}C$ for higher elevations or $\delta^{15}N$ for drier locales. For example, sub-fossil Nene sample five (NP.5) demonstrates an enriched $\delta^{13}C$ value (-15.1‰) perhaps due to the high elevation; however, NP.2 and NA.1 are nearly the same in value (both are –17.0‰), but they are from low elevation sites. We also see high elevation sources yielding depleted carbon isotope values (NA.3 at -22.2‰, NA.8 at –22.1‰). NP.5 also has a somewhat enriched $\delta^{15}N$ value (8.0‰), which may be due to the drier locale. This is most likely not the cause of the enrichment, since NP.2 is from a wet site and has yielded a higher $\delta^{15}N$ value (9.4‰).

Another environmental concern is soil age. In the Nene-only data set, there are samples from both Hawaii and Maui. Maui is an older island in the sequence, at approximately 1.4 mya, whereas Hawaii began forming about 0.5 mya and continues to be volcanically active (Carson and Clague, 1995). As noted earlier, Vitousek *et al.* (1989) found that increased soil maturity is associated with increased nitrogen isotope values. We would expect that the samples originating from Maui would have enriched $\delta^{15}N$ values. However, this pattern is not observed; the Maui samples are very similar to the other $\delta^{15}N$ values. This lack of differentiation supports the validity of isotopic comparison between the two islands, an important consideration for this study.

Sub-fossil Geese

The most striking feature of the sub-fossil geese data is the isotopic separation of the giant Hawaiian goose from the other two focus geese species (Figure 9). This group has lower carbon isotope values (-24.5 to –23.2‰) and somewhat depleted nitrogen isotope values (2.0 to 5.2‰). Statistical analysis demonstrated that the average isotope values for the giant Hawaiian geese are significantly different ($p<0.05$) from the sub-fossil Nene with which it co-existed, and in $\delta^{13}C$ ($p<0.05$) from the archeological Nene. The giant Hawaiian goose samples were from high elevations, two were dry sites (GG.1, GG.2) and the other two mesic (GG.3, GG.4). High elevations are expected to cause an increase in carbon isotope values, which is not demonstrated in this context (Vitousek, 1990). Thus, the isotopic separation must be due to another factor related to either the environment or diet. The depleted $\delta^{13}C$ values are likely due to the giant Hawaiian geese focusing almost entirely on C_3 plants, perhaps as much as 90% C_3. This greater selectivity may have resulted from two factors: a greatly reduced or even total absence of C_4 grasses in the habitat of the giant geese, or an evolutionary shift in diet to one that was less competitive with the co-existing Nene. The lower $\delta^{15}N$ values of the giant geese may be due to their consumption of depleted non-nitrogen-fixing plants in a closed-canopy environment.

The woods-walking goose and the Nene are indistinguishable from one another based on their isotopic composition. The woods-walking goose is morphologically intermediate between the Nene and giant Hawaiian goose. The latter has undergwent greater evolutionary change and became more highly specialized for the predator-free island environment. The woods-walking goose developed greater body size and near-complete or complete loss of flight capability. The lack of isotopic difference between the Nene and the woods-walking goose may have resulted from the lack of substantial evolutionary differentiation in diet between the two species. If that was the case, the two species may have been competitors with one another where their habitats overlapped in Maui. This question of competition between species is also a concern in modern avian studies, because many modern birds overlap in range and seem to have the same dietary niche, yet they are able to successfully co-exist. A detailed study of competition is difficult for extant species, but would be even harder to understand between extinct species, and in habitats and ecosystems no longer present.

Another feature of the sub-fossil geese data set is that the source island of the samples (Maui versus Hawaii) is not reflected in the carbon and nitrogen isotope values, as was also the case with the Nene. In the sub-fossil geese data set, all of the giant Hawaiian geese originated from Hawaii, the Woods-walking geese from Maui, and the Nene from both islands. An effect of soil age difference on isotope data is not supported in this instance because: 1) the $\delta^{15}N$ values of Hawaii samples are not uniformly low, and 2) the samples originating from Maui do not depart isotopically from the Hawaii samples.

Sub-fossil and Archeological Data

The complete data set of sub-fossil and archeological samples, including comparative species, was graphed to provide a broader comparison for isotopic analysis of the focus taxa (Figures 8 and 10). As discussed above, the most notable features of the geese data is that the Nene covers a broad range of isotopic values, the giant Hawaiian goose separates out isotopically, and the woods-walking goose is indistinguishable from the Nene.

The Dark-rumped Petrel (DP.1, DP.2) is a pelagic species that only comes to the islands to breed. They feed upon squid, fish and crustaceans (Hawaii Audubon Society, 1993). Both the carbon and nitrogen isotope composition of the petrels were highly enriched. This is a consequence of two factors: a marine diet and carnivory. Marine resources have an isotopic signature of high $\delta^{13}C$ and $\delta^{15}N$ values compared to most terrestrial environments (DeNiro and Epstein, 1981; Schoeninger and DeNiro, 1984). Additionally, carnivores have higher $\delta^{15}N$ values, being 3‰ enriched over their food source (DeNiro and Epstein, 1981; Peterson and Fry, 1987; Ambrose, 1991). The isotopic values of the two Dark-rumped Petrels seem to be entirely a result of their marine carnivorous diets. This result is expected given that petrels spend the majority of their lives at sea; the terrestrial influence would be very marginal, and would have little consequence on the isotopic composition of the petrel's tissues.

The feral pig (PI.1) was an omnivorous species that was indiscriminate in its dietary preferences, feeding upon both animal tissue and plant material. A carnivorous diet leads to enriched nitrogen isotope values, whereas an herbivorous diet lends depleted $\delta^{15}N$ values. The metabolic mixing of the two sources would lead to median nitrogen values, as seen in this sample. The carbon isotope value reflects a diet consisting of C_3 plants and C_4 grasses, or animals that ate a mixture of both.

The ibis (*Apteribis* sp., IB.1) was a flightless endemic to the Hawaiian archipelago that went extinct along with the woods-walking goose and giant Hawaiian goose (Olson and James, 1982b). When its ancestor arrived in the islands, specifically upon Maui Nui, it adapted to the terrestrial environment and began to change its diet away from marine and freshwater sources (James and Olson, 1991). The ibis may have been a forest browser, consuming invertebrates such as snails, land crabs and insects, and possibly berries. The diet and niche of this new form of ibis may parallel the New Zealand kiwis (Olson and James, 1991; H. James, personal communication). The isotopic composition of the ibis' collagenous bone protein suggests, however, that it may have been an herbivore, occupying a dietary niche similar to the Nene. This does support the contention that the ibis developed a new dietary niche concommitant with the evolution of a new body form and habitat niche. The flightless Hawaiian ibis still possessed a very long, decurved bill, a bill shape typically associated with probing into the soil to unearth grubs and other soil-dwelling organisms (Gill, 1995). Perhaps the flightless Hawaiian ibis specializeded upon roots, tubers, and fungi, which would be more consistent with its herbivorous isotopic signal.

Modern Ecosystem

The isotopic results for a small sub-set of the modern Hawaiian ecosystem demostrates a broad range in $\delta^{13}C$ and $\delta^{15}N$ values (Figure 11). The Nene have the lowest nitrogen isotope values, as would be expected for an herbivorous species. Modern Nene samples one and three (NM.1, NM.3) also have the lowest carbon isotope values. This is expected for a diet consisting entirely of C_3 plants, which was most likely true for these two individuals.

Columba livia (Rock Dove or feral Pigeon) is another herbivorous species, but it has different dietary preferences than the Nene, as reflected in the isotopic results. The Rock Dove consumes grains, small seeds, and fruit, and tends to live in close proximity to human populations (Robbins *et al.*, 1983). Both grains and fruit have enriched $\delta^{15}N$ values compared to nitrogen-fixing plants (Schoeller *et al.*, 1986), which is seen in the isotopic results. In addition, living near humans, the pigeons may have consumed proteinaceous foods while scavenging through our refuse, also lending and enriched $\delta^{15}N$ value.

The carnivorous species in this data set, the Pueo or Short-eared Owl (*Asio flammeus*), the cat (*Felis cattus*), and the Barn Owl (*Tyto alba*), all have enriched $\delta^{15}N$ values in comparison to the Nene. The Pueo, a secondary predator consuming insects, rodents and sometimes birds (Hawaii Audubon Society, 1993), displayed an enrichment amount in comparison to the modern nene isotope average that ranges from +1.62 to 4.68‰. It should be noted that Pueo sample one (PU.1, enrichment over Nene of 1.62‰) had an unusual C/N and collagenous protein yield (4.7 and 24.3%, respectively), which does not support isotopic indigeneity of the sample, thus the data of this sample should not be taken into consideration. Pueo sample three (PU.3) is more likely than sample two to have regularly taken young Nene as their meal, since it demonstrates greater than one trophic level enrichment over the Nene (+4.68‰). The cat and Barn Owl samples are similarly enriched, except for the feral cat sample two (CT.2). This sample demonstrates a +10‰ enrichment compared to the Nene, representing three trophic levels, which may be an artifact.

Conclusions

This stable isotopic study of Hawaiian avifauna has given insight into the paleoecology of a specialized endemic group of terrestrial geese, and allowed the dietary preferences of the three focus geese to be interpreted. The ancient Nene experienced a slight shift in diet after human colonization occurred. This may be due to the extreme habitat destruction and change in the lowlands, thereby preventing the Nene from continuing their seasonal elevational movements. The woods-walking goose was found to have a diet indistinguishable from the sub-fossil Nene. This result suggests that the two geese may have been occupying the same dietary niche, and may have been in competition with one another where their habitats overlapped. This conclusion, however, could be strengthened with a larger sample size of woods-walking geese. The giant Hawaiian goose was found to be isotopically distinct from the other sub-fossil geese, suggesting this species had evolved new dietary preferences. This change would have allowed the giant goose and Nene to co-exist in Hawaii without competing over food resources. Speciation events following colonization of the archipelago with a proto-typical large Canada Goose, therefore, appear to have resulted in habitat partitioning between some taxa, thus reducing competition.

The effect of several environmental conditions was explored in the isotopic data. Differences in elevation, soil age, and aridity tended not to shift the isotopic values. The condition of the habitat's foliage (*i.e.* closed-canopy versus open grassland) did seem to have a slight influence on the isotopic composition of the samples, although it was not easily separable. The majority of the isotopic variation has been attributed to dietary differences; specifically, the relative contribution of C_3 plants and C_4 grasses. The focus taxa in this study displayed a range of preferences from a predominantly C_3 to a more mixed diet.

A significant finding that may be of concern to the Nene Recovery Program is the evidence that a shift in the Nene's diet occurred after human colonization of the archipelago. This supports the contention that high elevation sites alone are not able to provide the Nene with all of their required nutrients. Low elevation localities must also be available to achieve a sustainable, healthy population.

TABLE 1: List of samples analyzed for focus geese taxa.

Sample ID	**Other ID**	**Taxon (common name)**	**Age BP**	**Locale**	**Elev.**	**Climate/Habitat**
NM.01.B1	MSU-8234a	*Branta sandvicensis* (Hawaiian Goose, Nene)	sub-fossil	Maui- Haleakala NP	high	dry/mesic, scrubby & sparse
NM.01.B2	MSU-8235a	"	sub-fossil	Maui- Haleakala NP	high	dry/mesic, scrubby & sparse
NM.01.B3	MSU-8236a	"	sub-fossil	Maui- Haleakala NP	high	dry/mesic, scrubby & sparse
NA.08.B1	AEC-99-5-4b	"	~275-500	Hawaii- Kihola	low	dry, barren lava field
NA.08.B2	AEC-99-5-5b	"	~210-400	Hawaii- Fireplough	2210	dry, open grassland
NA.08.B3	AEC-99-5-7b	"	sub-fossil	Hawaii- Hualalai Q.	5,500 ft.	dry
NA.08.B4	24896/1c	"	490 +- 60	H: Kihola Bay Cave	160	mesic, coastal
NA.08.B5	24896/2c	"	159 +- 60	H: Pu'u Keanui Cave	50	
NA.08.B6	24896/3c	"	325 +- 70	H: Pu'u Keanui Cave	50	
NA.08.B7	24896/4c	"	349 +- 60	H: Fireplough Cave	2210	dry, open grassland
NA.08.B8	24896/5c	"	267 +- 60	H: Fireplough Cave	2210	dry, open grassland
NA.08.B9	24896/6c	"	501 +- 60	H: Feather Cave		
NP.10.B1	AEC-99-5-10b	"	~800-2600	Hawaii- Umi'i Manu	4600	dry (above inversion)
NP.08.B2	AEC-99-5-11b	"	sub-fossil	Maui	low	mesic (60"/yr)
NP.08.B3	AEC-00-2-10b	"	sub-fossil	Maui- Lua Lepo	2710	mesic
NP.08.B4	26450/1c	"	~800-2600	Hawaii- Umi'i Manu	4600	dry, scrubby & open, bare lava
NP.08.B5	26450/2c	"	~800-2600	Hawaii- Umi'i Manu	4,600 ft.	dry, scrubby & open, bare lava
NP.08.B6	26450/3c	"	sub-fossil	Hawaii- Pohakuloa		
WG.08.B1*	AEC-99-5-12b	*Branta hylobadistes* (Woods-walking Goose)	sub-fossil	Maui- Pu'u Naio	1,000	arid leeward
WG.08.B2	AEC-00-2-9b	"	sub-fossil	Maui- Ulupalakal	high	mesic
WG.08.B3	AEC-00-2-11b	"	sub-fossil	Maui- Pu'u Naio	1,000	arid leeward
WG.08.B4	AEC-00-1-12b	"	sub-fossil	Maui- Pu'u Makua	high	mesic
GG.08.B1	AEC-99-5-2b	*Branta sp.* (Giant Hawaiian Goose)	sub-fossil	Hawaii- Haulalai, Pulani	5900	dry
GG.10.B2	AEC-99-5-8b	"	1645 +- 60	Hawaii- Umii Manu	4600	dry, scrubby & open, bare lava
GG.10.B3	AEC-99-5-9b	"	sub-fossil	Hawaii- Petrel Cave	4100 ft	mesic
GG.08.B4	AEC-00-2-2b	"	sub-fossil	Hawaii- Pulani	high	mesic

a: Sample source MSU Museum Collections, which were received as a gift from the Bernice P. Bishop Museum of Hawaii.

b: Sample source National Museum of Natural History (NMNH), Smithsonian Institution.

c: Sample analysis Rafter Radiocarbon Laboratory, Institute of Geological and Nuclear Sciences, New Zealand. Samples were provided by NMNH.

TABLE 2: List of samples analyzed for comparison purposes.

Sample ID	Other ID	Taxon (Common Name)	Age BP	Locale	Elev	Climate/Habitat
CT.08.B1	AEC-00-2-3b	*Felis catus* (feral cat)	<150	Hawaii- Kiholo	low	dry, lava tube
CT.08.B2	AEC-00-2-13b	"	<150	Hawaii- KaLae, Halii	200 ft.	0.25 m. inland, lava tube
OW.08.B1	AEC-00-2-5b	*Tyto alba* (Barn Owl)	modern	Hawaii- cave N. or Manuk	1840	
OW.01.B2	MSU-8240a	"	modern	Maui- unknown locale		
PU.01.B1	MSU-8237a	*Asio flammeus* (Pueo, Short-eared Owl)	modern	Maui- unknown		
PU.01.B2	MSU-8238a	"	modern	Maui- Hanaula Rd.		
PU.01.B3	MSU-8239a	"	modern	Maui- unknown		
RD.08.B1	AEC-00-2-4b	*Columba livia* (Rock Dove)	<150	Hawaii- Big S.Point Cave	656	urbanized
RD.08.B2	AEC-00-2-6b	"	<150	Hawaii- Big. S.Point Cave	656	urbanized
DP.08.B1	AEC-99-5-3b	*Pterodroma phaeopygia* (Dark-rumped Petrel)	~275-500	Hawaii- Kihola Q.	low	dry
DP.08.B2	AEC-99-5-6b	"	~210-400	Hawaii- Fireplaugh	2210 ft	dry, open grassland
PI.08.B1	AEC-99-5-1b	*Sus scrofa* (feral pig)	~275-500	Hawaii- Kihola Q.	low	dry, lava tube
IB.08.B1	AEC-00-2-7b	*Apteribis sp.* (flightless Hawaiian ibis)	1710 +- 60	Maui- Ulupakala, Pu'u Ma	4800	mesic

a: Sample source MSU Museum Collections, which were received as a gift from the Bernice P. Bishop Museum of Hawaii.

b: Sample source National Museum of Natural History (NMNH), Smithsonian Institution.

c: Sample analysis Rafter Radiocarbon Laboratory, Institute of Geological and Nuclear Sciences, New Zealand. Samples were provided by NMNH.

TABLE 3: Collagenous protein yields, C to N ratios, C and N isotope values for focus geese taxa.

Sample ID	**Taxon**	**% Yields**	**C/N**	**∂15N (%)**	**∂13C (%)**
NM.01.B1	*Branta sandvicensis* (Hawaiian Goose, Nene)	8.4	—	6.47	-25.80
NM.01.B2	"	19.9	—	6.87	-15.60
NM.01.B3	"	14.0	—	6.14	-23.90
NA.08.B1	"	14.0	3.3	5.98	-17.00
NA.08.B2	"	17.0	—	6.04	-19.90
NA.08.B3	"	20.0	3.1	0.53	-22.21
NA.08.B4	"	17.2	3.1	12.90	-14.30
NA.08.B5	"	16.0	3.1	6.10	-22.70
NA.08.B6	"	19.4	3.1	5.20	-19.50
NA.08.B7	"	19.4	3.1	3.10	-20.40
NA.08.B8	"	18.0	3.2	2.30	-22.10
NA.08.B9	"	18.5	3.2	6.70	-21.30
NP.10.B1	"	16.2	3.0	6.33	-21.28
NP.08.B2	"	12.2	3.2	9.41	-17.00
NP.08.B3	"	14.7	3.4	4.09	-18.68
NP.08.B4	"	—	3.0	7.70	-18.60
NP.08.B5	"	—	3.1	8.00	-15.10
NP.08.B6	"	—	3.0	7.40	-21.70
WG.08.B2	*Branta hylobadistes* (Woods-walking Goose)	17.9	3.0	7.15	-20.37
WG.08.B3	"	18.7	—	—	-18.57
WG.08.B4	"	15.9	3.0	5.85	-19.55
GG.08.B1	*Branta sp.* (Giant Hawaiian Goose)	6.4	3.5	1.99	-24.47
GG.10.B2	"	10.2	3.2	2.32	-23.15
GG.10.B3	"	2.2	3.6	2.95	-25.91
GG.08.B4	"	3.5	3.8	5.19	-23.79

TABLE 4: Collagenous protein yields, C to N ratios, and C and N isotope values for comparison taxa.

Sample ID	Taxon	% Yields	C/N	∂15N (%)	∂13C (%)
CT.08.B1	*Felis catus* (feral cat)	17.4	3.0	10.00	-13.68
CT.08.B2	"	16.5	3.1	16.55	-12.59
OW.01.B2	*Tyto alba* (Barn Owl)	13.3	3.4	11.42	-10.07
OW.08.B1	"	20.0	3.1	10.22	-19.54
PU.01.B1	*Asio flammeus* (Pueo, Short-eared Owl)	24.3	4.7	8.12	-17.84
PU.01.B2	"	17.2	3.5	8.87	-16.42
PU.01.B3	"	15.0	3.1	11.18	-14.86
RD.08.B1	*Columba livia* (Rock Dove)	18.4	3.1	12.61	-20.42
RD.08.B2	"	21.1	3.1	12.35	-20.89
DP.08.B1	*Pterodroma phaeopygia* (Dark-rumped Petrel)	16.4	3.1	16.08	-12.27
DP.08.B2	"	16.4	3.1	15.15	-12.58
PI.08.B1	*Sus scrofa* (feral pig)	15.3	3.2	13.28	-15.66
IB.08.B1	*Apteribis sp.* (flightless Hawaiian ibis)	16.2	2.9	6.40	-19.36

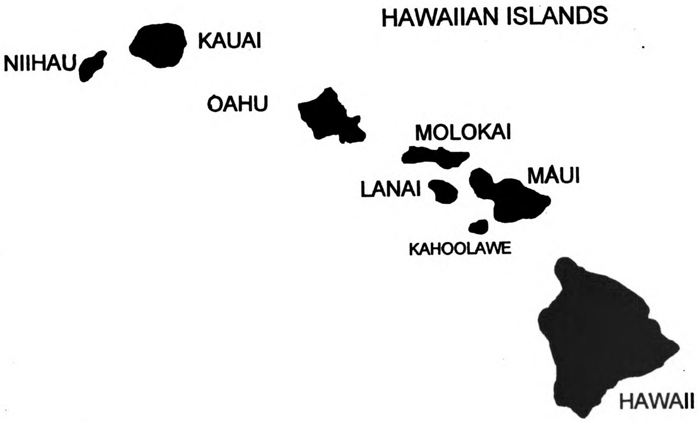


FIGURE 1: The Hawaiian Archipelago (Pratt *et al.*, 1987).

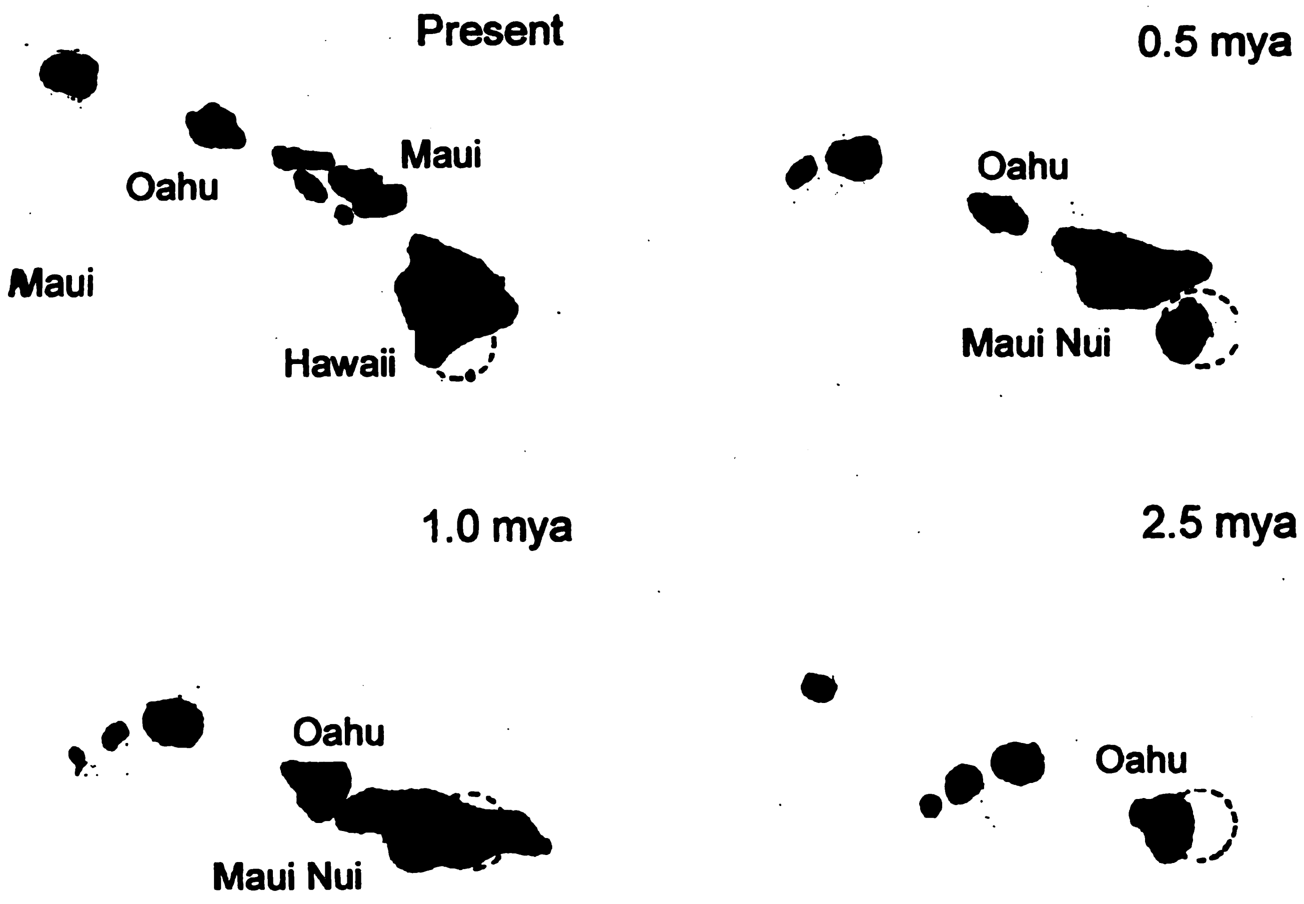


FIGURE 2: Time sequence of the formation of the Hawaiian Archipelago, including Maui Nui (Carson and Clague, 1995).

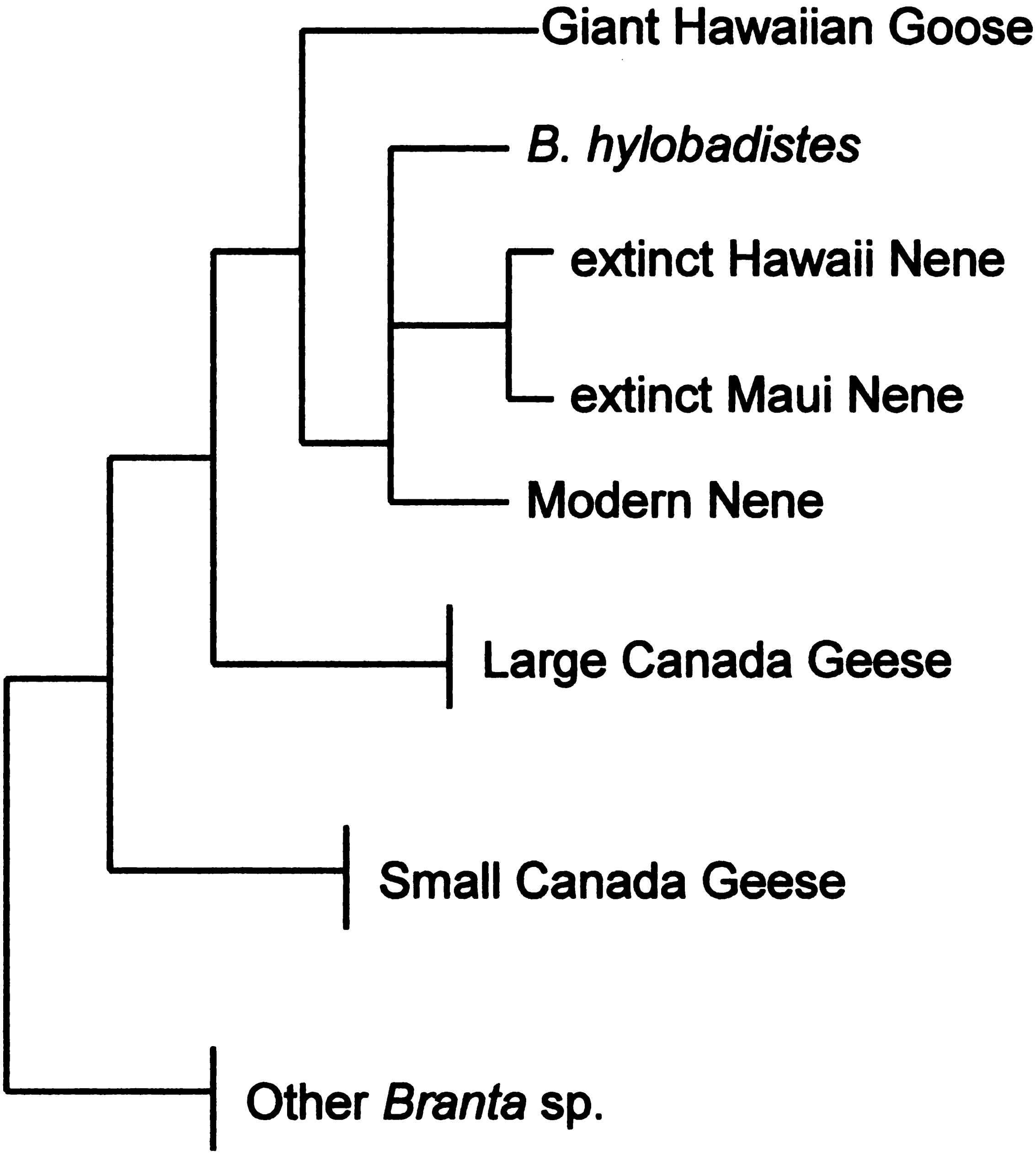


FIGURE 3: Cladogram of Hawaiian geese (Paxinos, 1998).

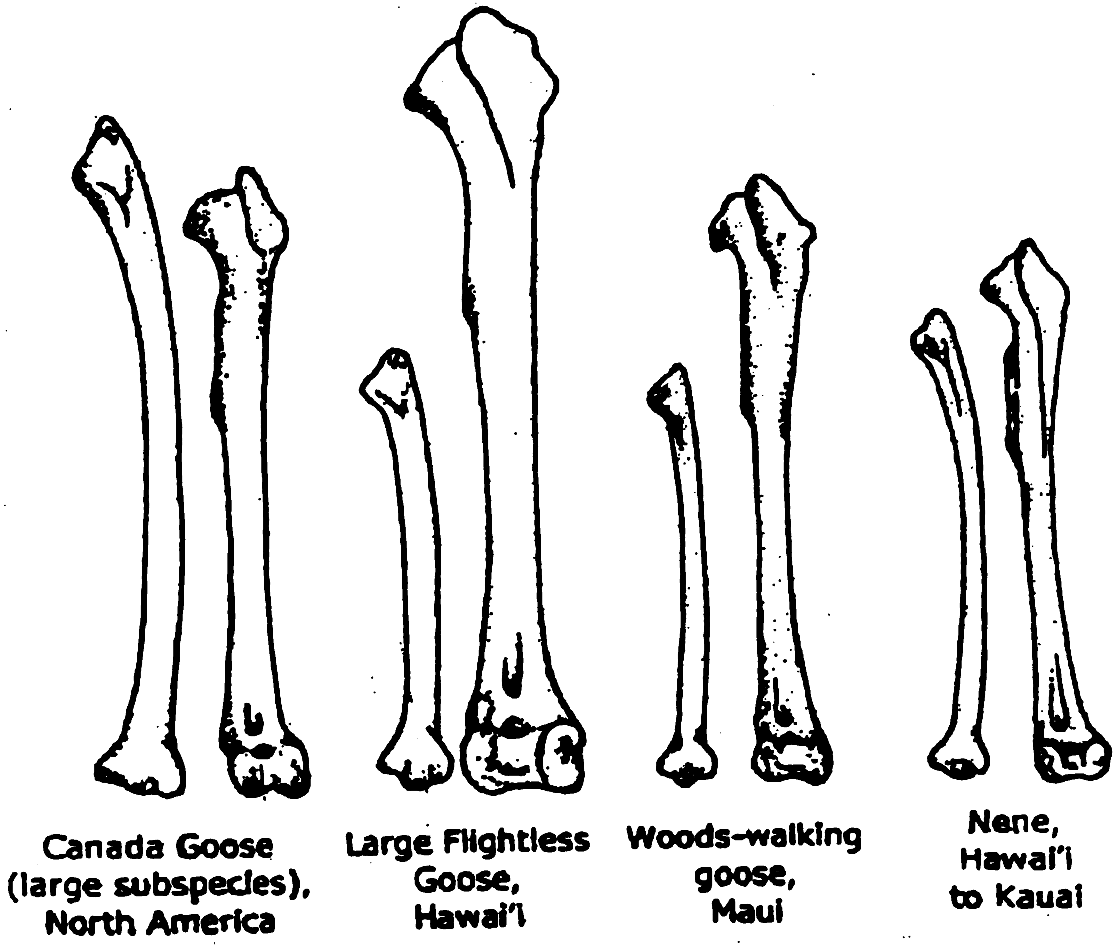
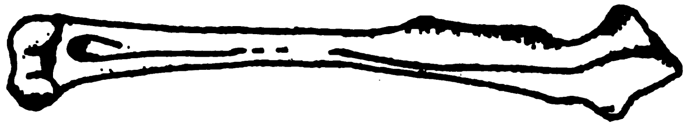


FIGURE 4: Limb proportions in the Hawaiian radiation of true geese (*Branta*). Comparison of wing (radius) and leg (tibiotarsus) bones.

Methodology



(Cleaned manually and by sonication)

↓ Powderized

Pre-Wash

(Na-buffer to remove humic acids)

↓ Centrifuged

Demineralization

(1N HCl, 4 C, approx. 18 hr.s)

↓ Centrifuged

Dialysis

(5 days, 4°C, distilled H_2O, 3500 MWCO tubing)

↓ Freeze-dried

Geochemical Analyses

(Protein yield, C/N, isotopic)

Figure 5: Summary of methodology.

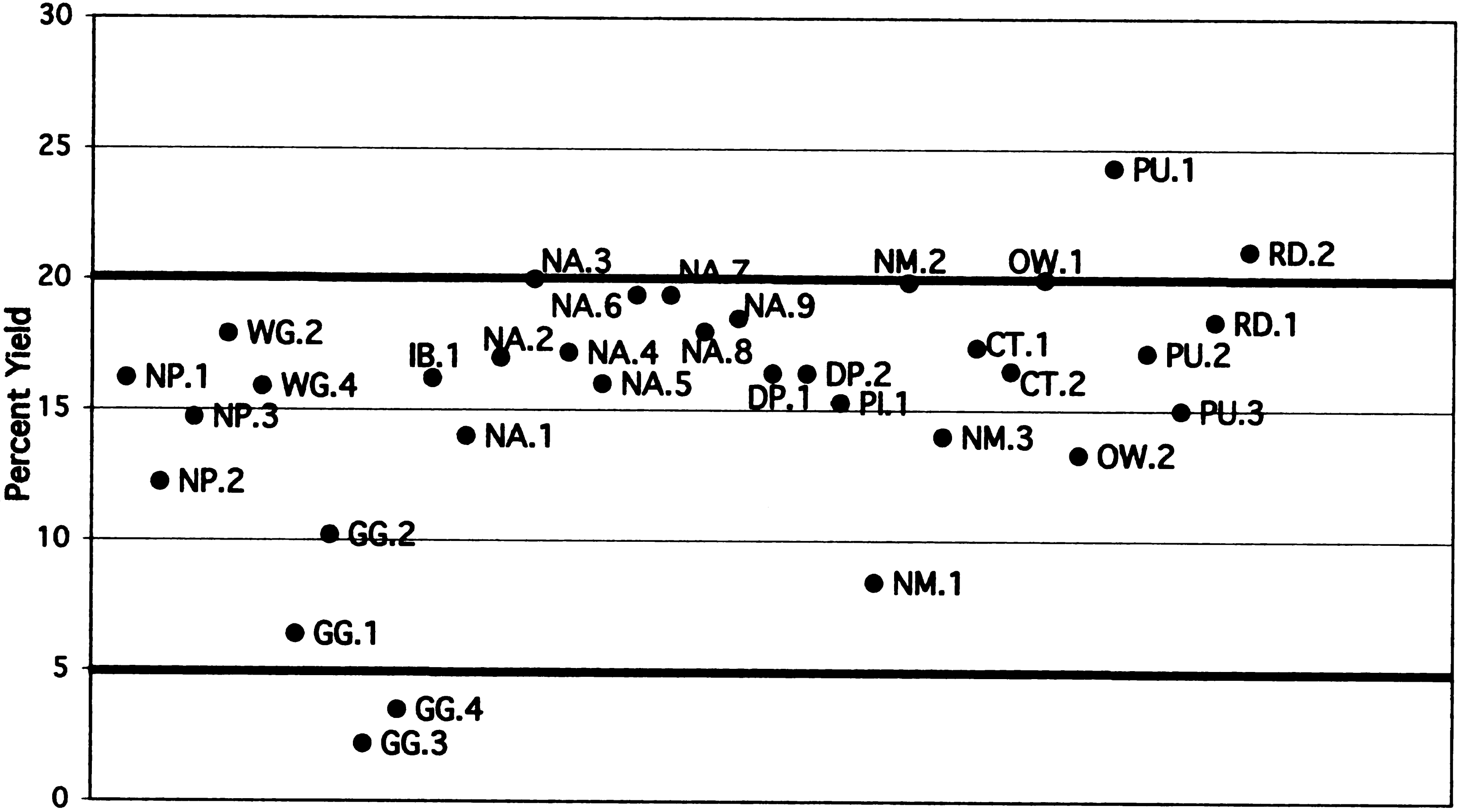


FIGURE 6: Collagenous Protein Yields (% by Weight)
Key to abbreviations is provided in Tables 1 and 2.

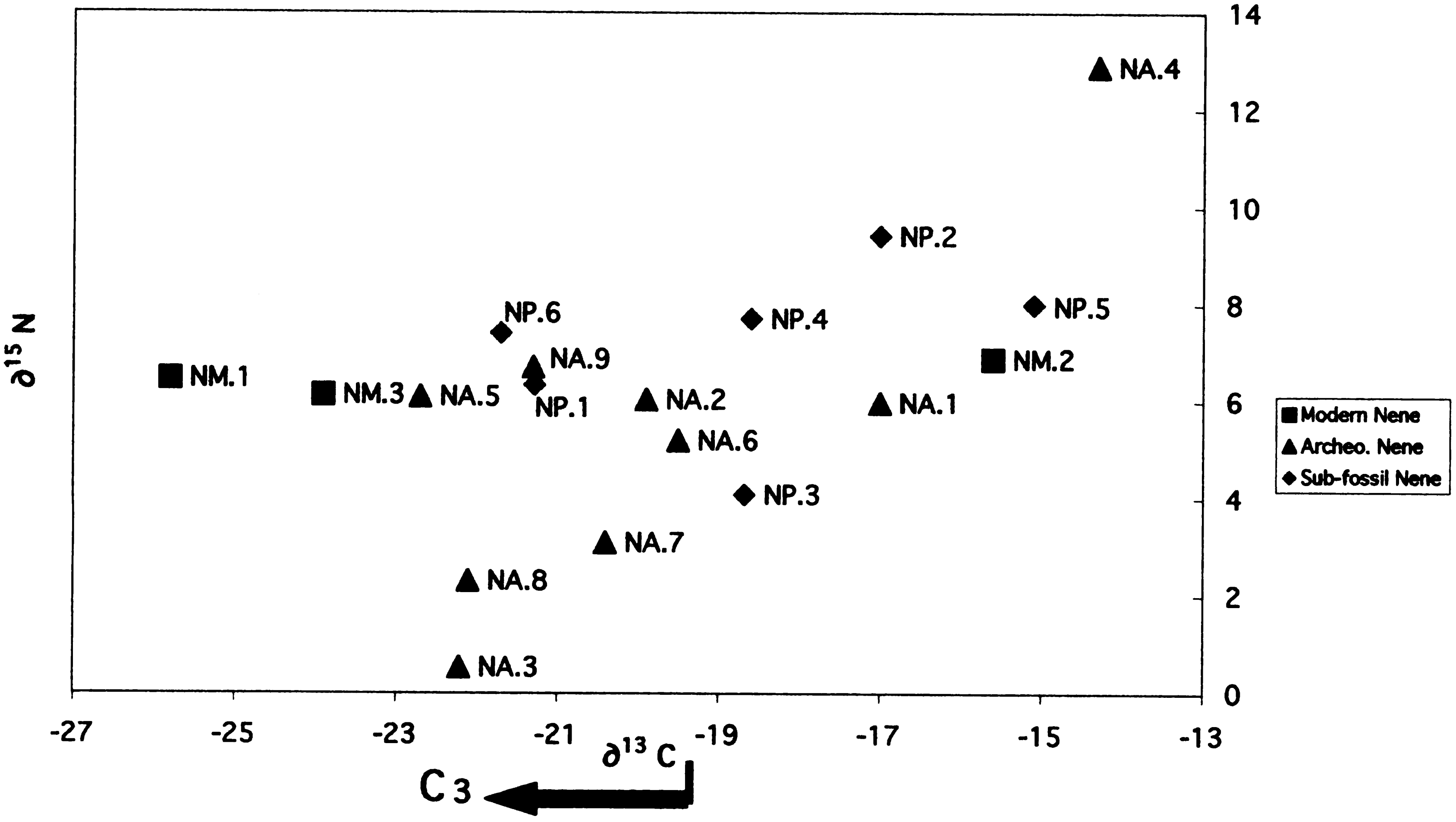


FIGURE 7: Nene Isotopic Results
Key to abbreviations is provided in Tables 1 and 2.

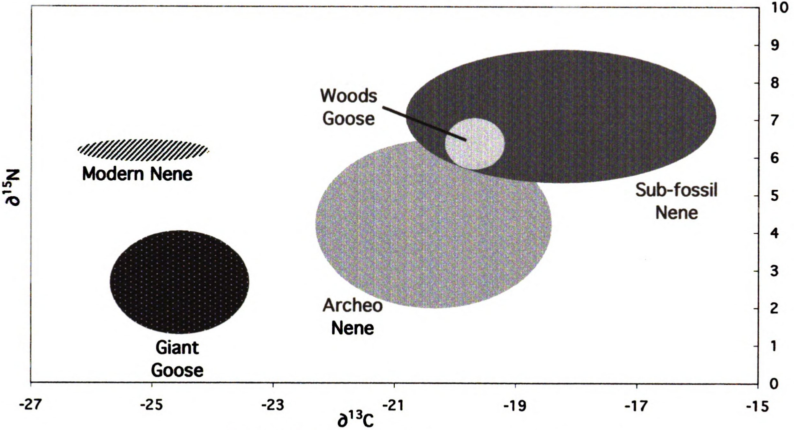


FIGURE 8: Averaged focus geese isotopic results

Key to abbreviations is provided in Table 1 and 2.

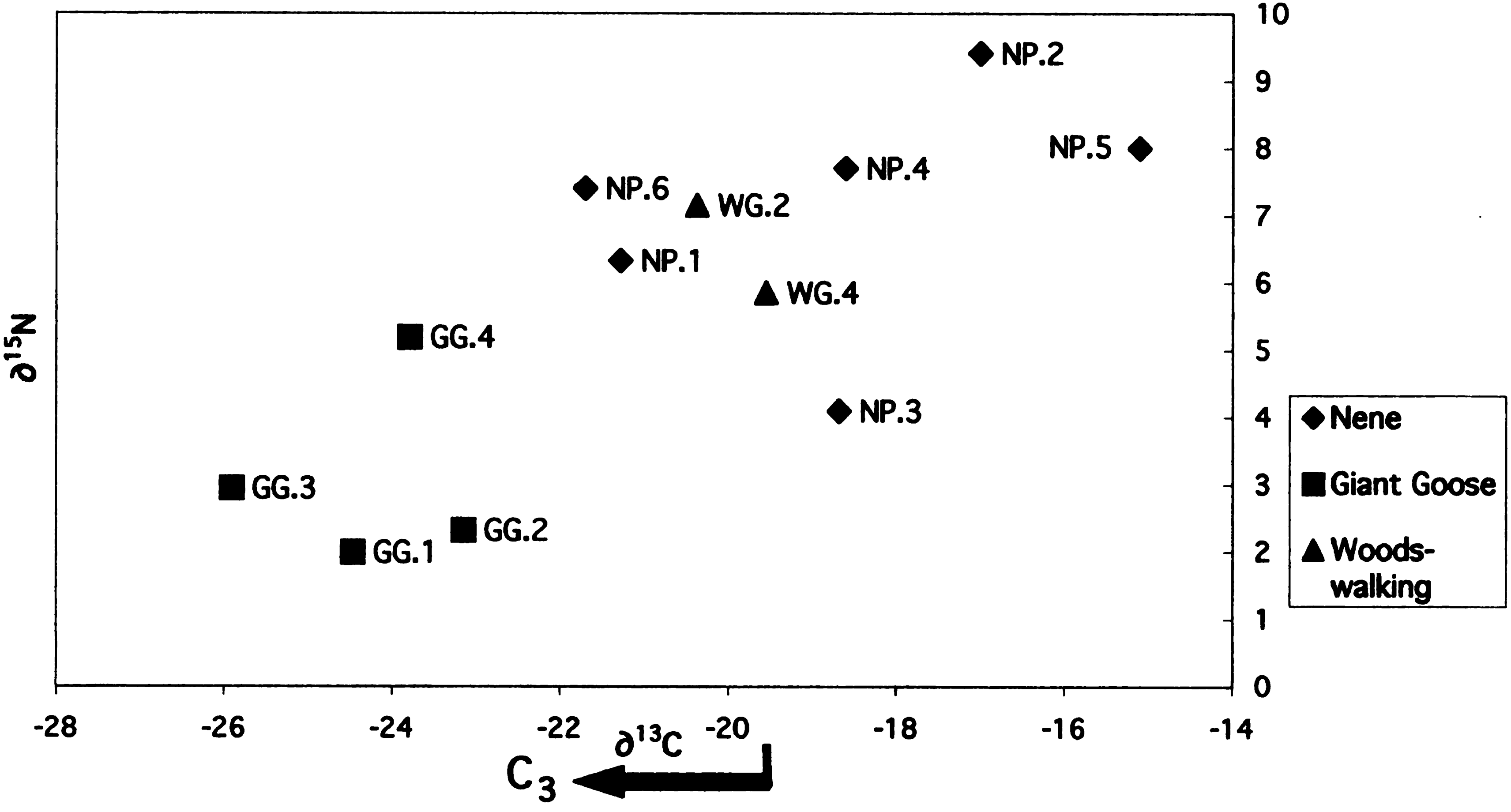


FIGURE 9: Sub-fossil Geese Isotopic Results
Key to abbreviations is provided in Tables 1 and 2.

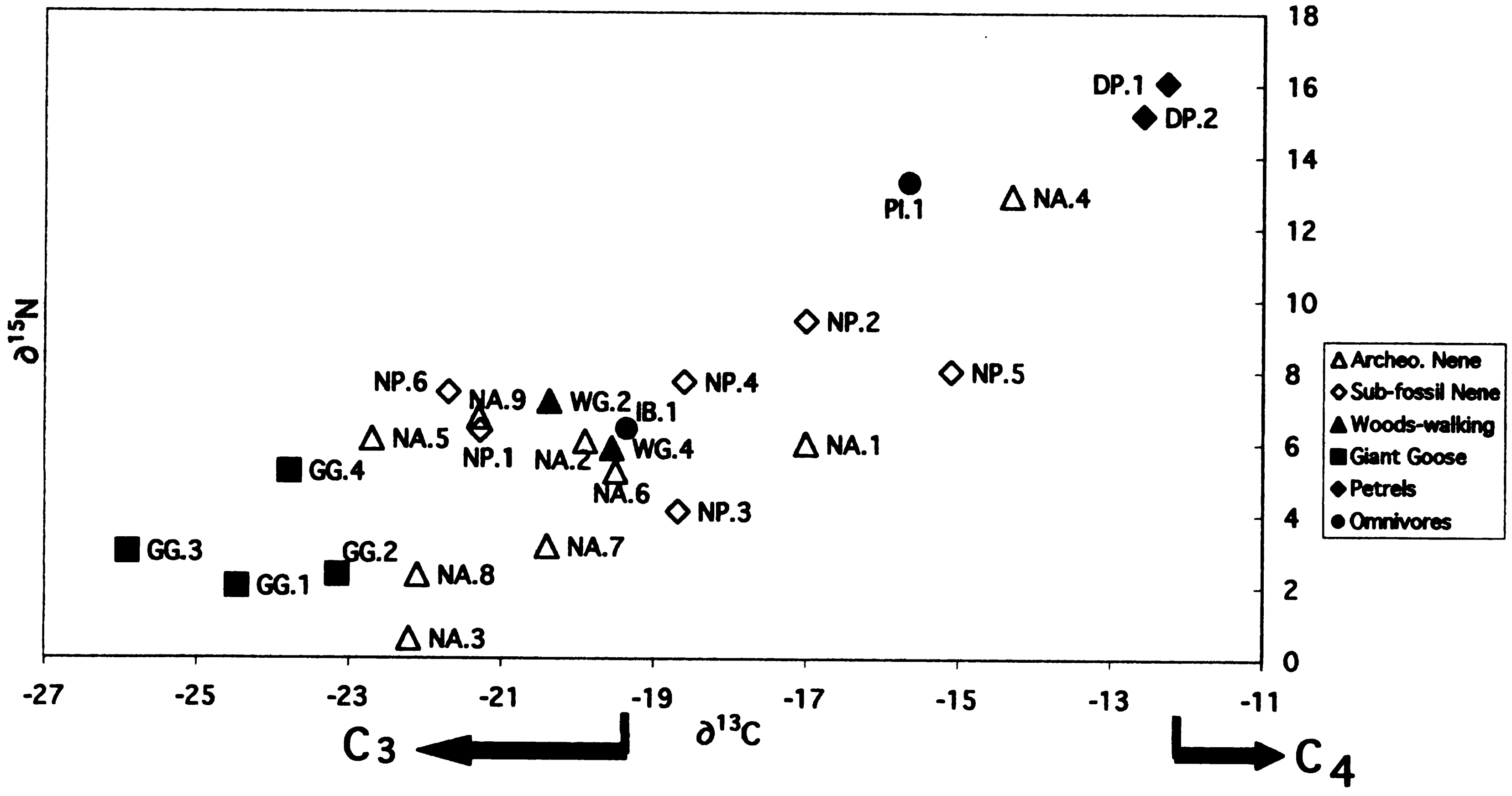


FIGURE 10: Sub-fossil and Archeological Isotopic Results
Key to abbreviations is provided in Tables 1 and 2.

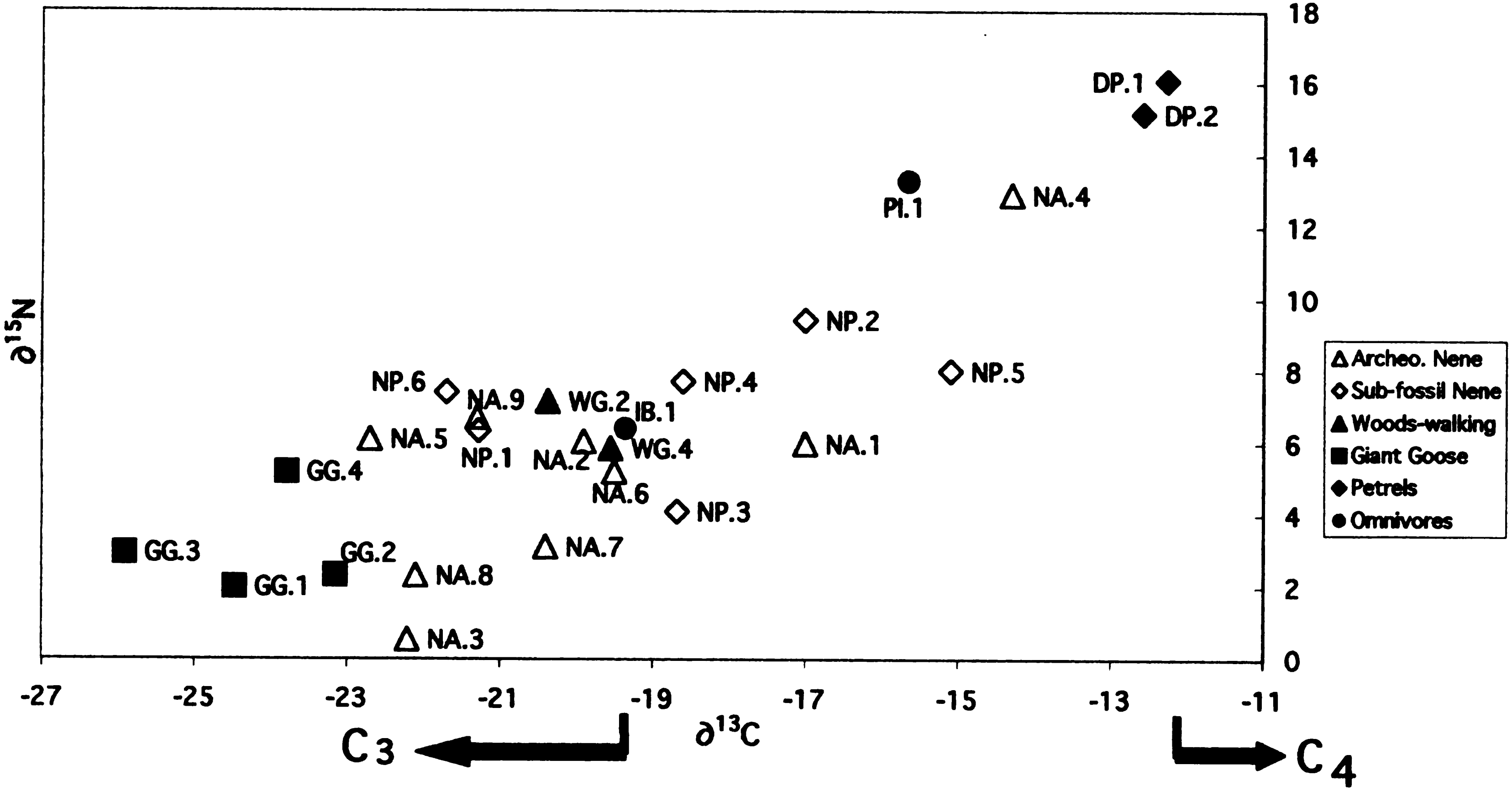


FIGURE 10: Sub-fossil and Archeological Isotopic Results
Key to abbreviations is provided in Tables 1 and 2.

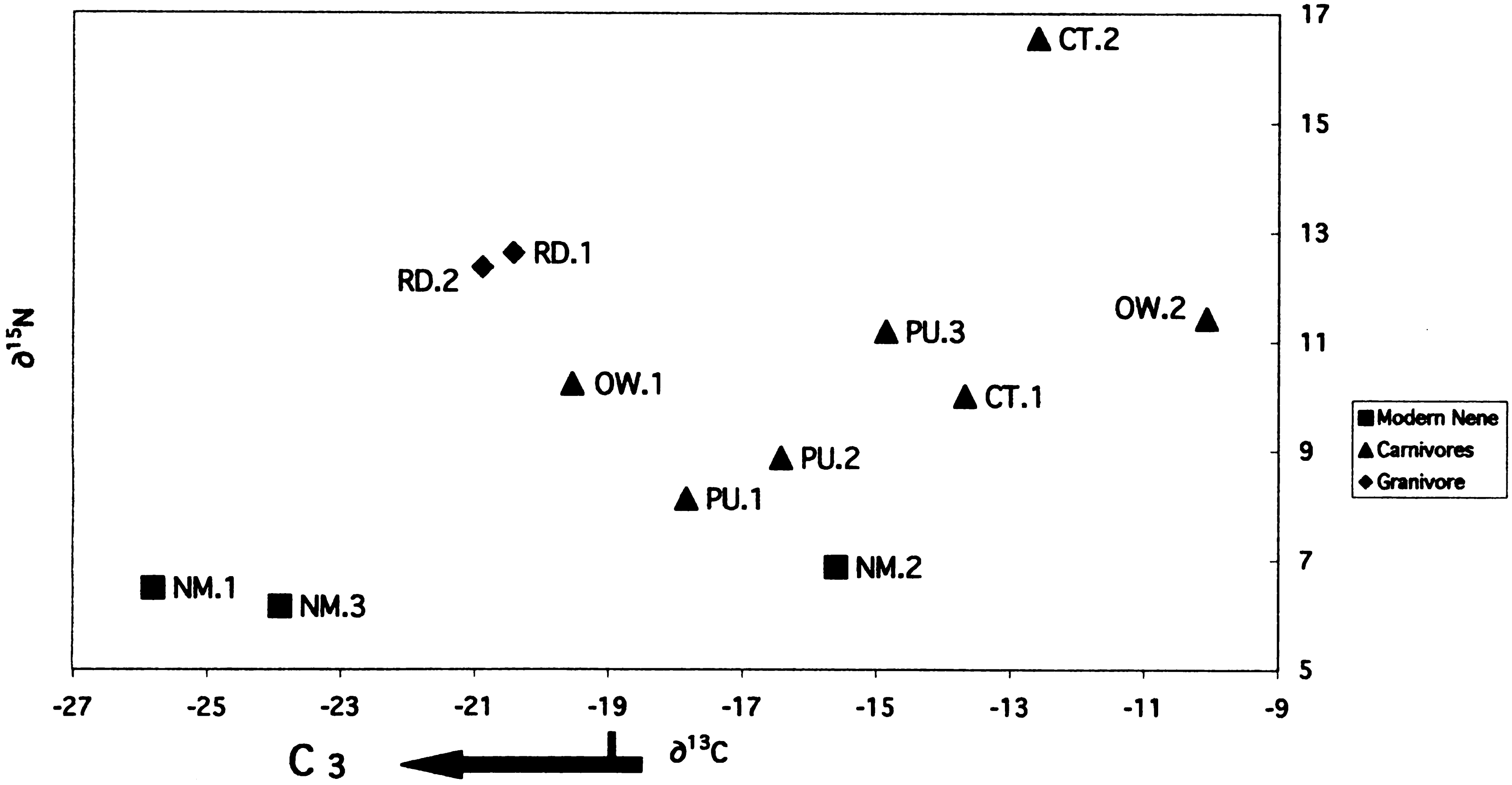


FIGURE 11: Modern ecosystem isotopic results
Key to abbreviations is provided in Tables 1 and 2.

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