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Isotopic Analysis of ***Wolf NY201998*** indicates a diet typical of naturally wild NY canids, not like a captive/pet.

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Much of the theory for this analysis is based on a previous study by Dr. Roland Kays and myself (reference below). I will outline some of the background for the analysis below using excepts from that study:

Kays, R.W., and **Feranec, R.S.** *2011*. Carbon isotopes distinguish wild from domestic animals in C_3 environments: a study with eastern wolves. ***Northeast Naturalist 18: 253-264.***

Background

Carbon and Nitrogen Isotopes

Stable carbon and nitrogen isotopes obtained from the tissues of animals have proven beneficial in distinguishing domestic from wild individuals (Barton et al., 2009; Hu et al., 2009; Minagawa et al., 2005; Noe-Nygaard et al., 2005). The analysis of carbon isotope values (δ13C values) is useful for examining differences in diet because the different photosynthetic pathways used by plants (e.g., C_3 pathway, C_4 pathway) impart different $^{13}C/^{12}C$ ratios to plant tissues, and animals consuming those plant tissues, or predators of those animals, will reflect the ratio ingested. In northeastern USA, most plants use the C_3 photosynthetic pathway (Barton et al., 2009; Hu et al., 2009; Minagawa and Wada, 1984; Noe-Nygaard et al., 2005; Sage et al., 1999b), which have a mean isotope value of -27.0‰ and typically range from –22‰ to –35‰. In contrast, plants using the C_4-photosynthetic pathway have a mean isotopic value of -13.0‰ and generally range from -9‰ to –19‰ (Ehleringer and Monson, 1993; Ehleringer et al., 1991; Farquhar et al., 1989; Koch, 1998; O'Leary, 1988). While C_4 plants are not naturally abundant on the landscape in northeastern North America (Sage et al., 1999b), corn (*Zea mays*) is a non-native C_4 plant that is important in the diet of many domestic animals either as a direct component of their diet (e.g. corn-based pet food) or as grain feed for domestic stock which are then fed to predators. Therefore, in environments dominated by native C_3 plants we predict wild animals to show more negative carbon isotope values compared with less negative values in captive animals eating a domesticated diet.

Similar to carbon isotopes, nitrogen isotope values in mammals are a function of what is ingested (Ambrose, 1991; DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Sealy et al., 1987). For carnivores, $\delta^{15}N$ values will mainly be affected by their trophic position, whether fertilizer was incorporated into prey foods, and certain ecological conditions (Ambrose, 1991; DeNiro and Epstein, 1981; Fox-Dobbs et al., 2007; Heaton, 1986; Minagawa and Wada, 1984; Roth and Hobson, 2000; Sealy et al., 1987). Nitrogen isotopes fractionate up the food chain such that predators generally have $\delta^{15}N$ values 3-5‰ more positive than their food (Ambrose, 1991; Koch, 1998). Additionally, carnivores that incorporate commercially fertilized foods or prey whose foods have been commercially fertilized will tend to have more negative $\delta^{15}N$ values (DeNiro and Epstein, 1981; Heaton, 1986; Minagawa and Wada, 1984). Within ecosystems, animals will tend to display more positive $\delta^{15}N$ values in areas that are warm and arid (Ambrose and DeNiro 1986a, 1987, Ambrose 1991, Amundson et al. 2003 Heaton et al. 1986, Gröcke et al. 1997, Koch 1998, Schwarcz et al. 1999, Robinson 2001). Finally, N_2-fixation can also influence $\delta^{15}N$ values as well. Prey that predominantly forage N_2-fixing plants will tend to have more negative $\delta^{15}N$ values compared to prey feeding on plants that do not fix N_2 (Ambrose 1991, Koch 1998).

As you move up the food chain, there is a discrimination of isotope values as that food is consumed—typically isotope values get higher (more positive). Because of this, we adjusted the $\delta^{13}C$ and $\delta^{15}N$ values to compare the results of **Wolf NY201998** more directly to their food sources. To make the correction, we converted isotope values using isotopic discriminations for both collagen and hair. Collagen has been shown to be +5.0‰ enriched in $\delta^{13}C$ from food, while the $\delta^{15}N$ value was +3.0‰ enriched (Schoeninger and Deniro, 1984; Minegawa and Wada, 1984). For hair samples we follow Roth and Hobson (2000) who showed an enrichment in canid $\delta^{13}C$ values of +2.6‰ from consumed food, while $\delta^{15}N$ was +3.2‰.

Tissues

The different tissue types develop over different periods of time in an individual's life. Because of this, the isotopes found in the different tissues represent the life history during the incorporation of those isotope values. Three different tissues were sampled for **Wolf NY201998**. Collagen from the tooth root will represent isotopic incorporation prior to about 6 months of age. Collagen from the nasal bones represent isotopic incorporation as an average of the lifetime of the individual. Hair will represent recent incorporation of isotopic values prior to the last molt. So, these tissues will provide us with a picture of diet from early in life (tooth root), average life (nasal bones), and late life (hair).

Methods:

The tooth root and nasal bones were treated using the bone protocol of the University of California, Irvine lab that provided the isotopic analyses:
https://bpb-us-e2.wpmucdn.com/sites.uci.edu/dist/1/2856/files/2016/12/bone_protocol.pdf

Acid-Base-Acid Protocol mentioned for the hair sample can be found here:
https://bpb-us-e2.wpmucdn.com/sites.uci.edu/dist/1/2856/files/2016/12/aba_protocol.pdf

Specific Comments on sample preparation:

- The wolf hair sample was treated with acid-base-acid, but with a weak base step (0.05N NaOH at rm temp.) to avoid protein loss.
- The bone and tooth samples were sonicated in solvents at ~40°C to remove lipids: 2:1 chloroform/methanol (2hrs), methanol (1hr) and MQ water (1hr).
- They were then decalcified in 1N HCl, gelatinized at 60°C and pH 2, and ultrafiltered to select a high molecular weight fraction (>30kDa).

Results

Table 1. Isotope results for **Wolf NY201998.** %C, %N and C/N are used to assess quality of the collagen. The values fall in the range for modern mammals and indicate accurate analysis. Values presented in the table below are the original values. That is, not adjusted by trophic enrichment factors (TEFs).

Sample	**$\delta^{15}N$ (‰)**	**$\delta^{13}C$ (‰)**	**%N**	**%C**	**C/N (atomic)**
NY201998A wolf bone	8.8	-20.3	16.5	45.5	3.2
NY201998B wolf tooth	5.2	-21.8	16.7	44.8	3.1
NY201998C wolf hair	7.5	-21.7	15.4	46.0	3.5

The figures below show the isotope values for domestic food, wild food, captive canids, urban canids, and wild canids as well as isotope values for tissues of specific canids from the northeast USA (Fig 1, 2). The carbon isotope values of the bone and/or hair of NY 08 (NYSM ZM 15631) and NY 05 (NYSM ZT-161; Fig 1B) are high and are indicative of animals being fed pet food in a captive environment. NY 01 (NYSM ZM 14276) has lower values indicative of an individual that had a typical wild diet of terrestrial animals living in NY. Interestingly, ME 96 (MCZ 62507) had collagen values (earlier in life) that indicate a high percentage of corn in the diet (high $\delta^{13}C$ values), while the hair values of this same individual indicate consumption of a typical wild canid diet. We interpret ME 96 (MCZ 62507) as having been a released pet. **Wolf NY201998** has isotope values the same as NY 01 (NYSM ZM 14276), and both are interpreted as having a typical diet of a wild individual canid in NY.

The nitrogen isotope values (Fig 2) for **Wolf NY201998** fall within the range observed for a typical terrestrial diet from NY.

Figures

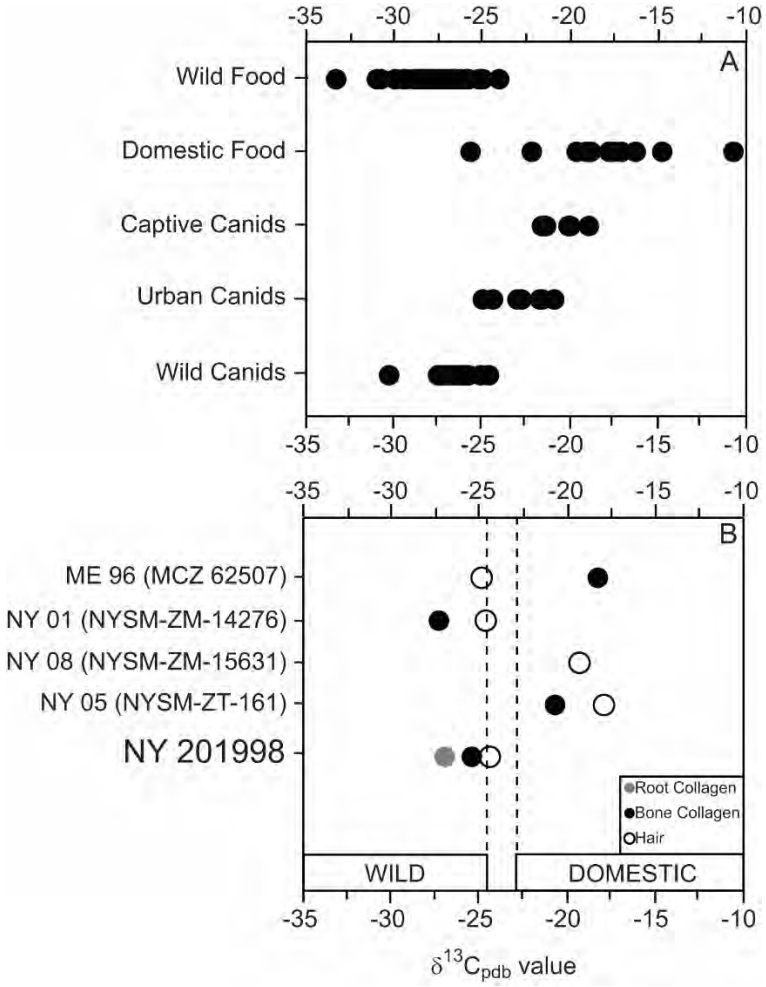


Figure 1. Stable carbon isotope values for canids and their prey. Isotope values of NY201998 indicate a diet typical of wild canids. Values for NY201998 have been adjusted based on their tissue type (see text).

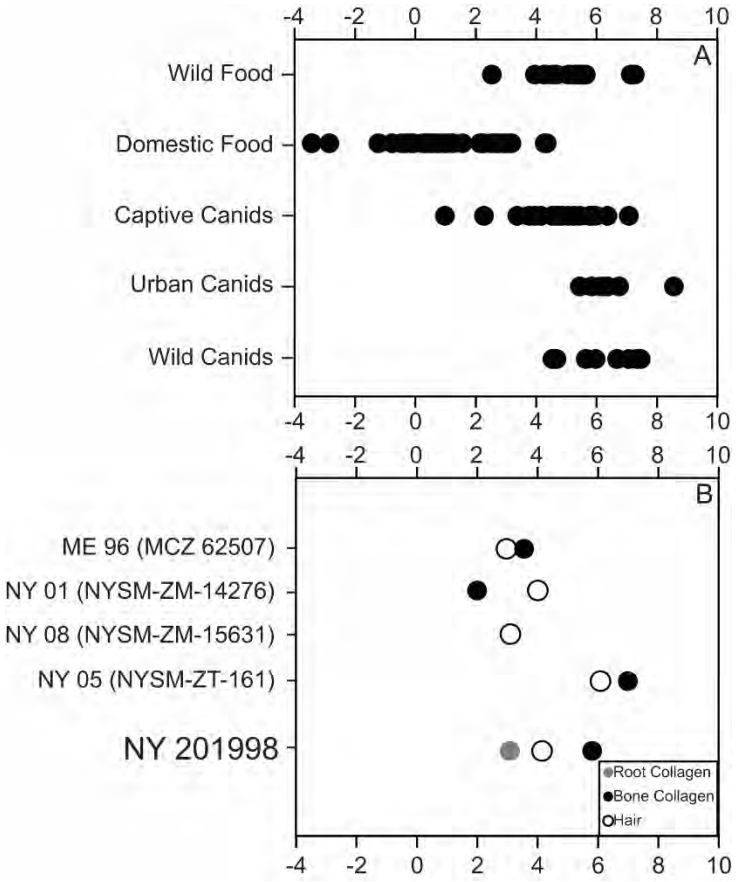


Figure 2. Stable nitrogen isotope values for canids and their prey. Isotope values of **Wolf NY201998** fall within the range for a species with a terrestrial diet—not marine fish. Nitrogen isotope values have not been found to be able to conclusively discriminate between a captive terrestrial diet and a wild terrestrial diet. Values for **Wolf NY201998** have been adjusted based on their tissue type (see text).

Interpretation:

- **Wolf NY201998 has isotope values that are typical for a wild NY canid.**

In my opinion, the isotopic values indicate incorporation from typical wild food sources, not from pet food or anthropogenic foods (garbage waste consumption) for ALL three analyzed tissues. **Wolf NY201998** does not appear to have been a pet or have been consuming significant human food through eating garbage, for example.

Interestingly, the hair carbon isotope value does fall just within the "ambiguous" area on the graph (fig 1B). However, the data point is within the margin of error for the isotopic analysis (0.2 permil for N) of indicating a typical wild diet. Combined with the collagen isotopic values I do not believe a significant change in diet occurred since the last molt compared to the average diet of this individual—consuming isotopic values typical of dietary resources of a wild animal in the Northeast USA.

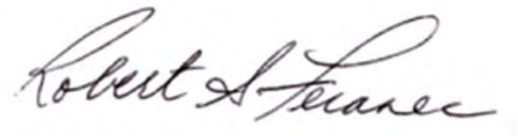
Analysis of carbon isotope values appears to distinguish consumption of pet foods that have corn as an ingredient as well as "wild" animals that are consuming human food waste through garbage---both of which have a high C_4 signal (i.e., corn signal) and thus results in higher $\delta^{13}C$ values. For **Wolf NY201998**, all carbon values, when adjusted for their trophic enrichment factors, indicate a wild diet (i.e.,

consuming prey that consumed predominantly C_3 plants or consuming C_3 plants directly) typical for wild NY canids.

Nitrogen isotopes are less diagnostic for determining whether a canid was wild or a pet, but the values obtained from the three tissue types do not indicate consumption of food that had a large percentage of commercially fertilized corn as an ingredient (values near zero) or indicate a diet of fish (higher N isotope values, likely greater than 10).

Please let me know if you have any questions or need any clarifications.

Sincerely,



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