Ancestors of domestic cats in Neolithic Central Europe: Isotopic evidence of a synanthropic diet

Magdalena Krajcarz[a,1], Maciej T. Krajcarz[b], Mateusz Baca[c], Chris Baumann[d,e,f], Wim Van Neer[g,h], Danijela Popović[c], Magdalena Sudoł-Procyk[a], Bartosz Wach[b], Jarosław Wilczyński[i], Michał Wojenka[j], and Hervé Bocherens[e,f]

[a]Institute of Archaeology, Nicolaus Copernicus University in Toruń, 87-100 Toruń, Poland; [b]Institute of Geological Sciences, Research Centre in Warszawa, Polish Academy of Sciences, 00-818 Warszawa, Poland; [c]Centre of New Technologies, University of Warsaw, 02-097 Warszawa, Poland; [d]Institute for Scientific Archaeology, University of Tübingen, 72070 Tübingen, Germany; [e]Senckenberg Centre for Human Evolution and Palaeoenvironment, University of Tübingen, 72076 Tübingen, Germany; [f]Biogeology, Department of Geosciences, University of Tübingen, 72074 Tübingen, Germany; [g]Operational Direction Earth and History of Life, Royal Belgian Institute of Natural Sciences, B-1000 Brussels, Belgium; [h]Laboratory of Biodiversity and Evolutionary Genomics, University of Leuven, B-3000 Leuven, Belgium; [i]Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, 31-016 Kraków, Poland; and [j]Institute of Archaeology, Jagiellonian University, 31-007 Kraków, Poland

Edited by Melinda Zeder, National Museum of Natural History, Santa Fe, NM, and approved June 4, 2020 (received for review November 20, 2019)

Cat remains from Poland dated to 4,200 to 2,300 y BCE are currently the earliest evidence for the migration of the Near Eastern cat (NE cat), the ancestor of domestic cats, into Central Europe. This early immigration preceded the known establishment of housecat populations in the region by around 3,000 y. One hypothesis assumed that NE cats followed the migration of early farmers as synanthropes. In this study, we analyze the stable isotopes in six samples of Late Neolithic NE cat bones and further 34 of the associated fauna, including the European wildcat. We approximate the diet and trophic ecology of Late Neolithic felids in a broad context of contemporary wild and domestic animals and humans. In addition, we compared the ecology of Late Neolithic NE cats with the earliest domestic cats known from the territory of Poland, dating to the Roman Period. Our results reveal that human agricultural activity during the Late Neolithic had already impacted the isotopic signature of rodents in the ecosystem. These synanthropic pests constituted a significant proportion of the NE cat's diet. Our interpretation is that Late Neolithic NE cats were opportunistic synanthropes, most probably free-living individuals (i.e., not directly relying on a human food supply). We explore niche partitioning between studied NE cats and the contemporary native European wildcats. We find only minor differences between the isotopic ecology of both these taxa. We conclude that, after the appearance of the NE cat, both felid taxa shared the ecological niches.

wildcat | synanthropic species | stable isotopes | paleoecology | trophic niche

Many new relationships between humans and animals began in the Neolithic Period when agriculture emerged in human societies. Major steps of those times included the domestication and husbandry of animals. Domestication is seen as a process where people take an animal population into direct and active management (1, 2). In general, domestication leads to relationships where both partners (i.e., domesticated animals and their human masters) gain certain benefits (1). It can be characterized either from an economic perspective (humans taking control over animals to increase resource) or by the way animals become integrated into the social structure of human society (1–3).

Some wild animal species benefit from living in human-made environments without coming into direct contact with people. They benefit by simply exploiting anthropogenic habitats (rural, agricultural, or urban) for food or shelter (4–7). In animal ecology, such behavior is categorized as synanthropy. We follow O'Connor (6) and Johnston (7), who defined synanthropes as organisms that cohabit with humans and live in or around human-modified or human-made environments. Synanthropy includes a wide range of degrees, from human-dependent full synanthropes to opportunistic and occasional synanthropes (7, 8) (details are provided in *SI Appendix*, Fig. S1). In general, synanthropy includes many types of ecological interactions with humans (9, 10). Among them, commensalism played a particularly important role in domestication of carnivores. We use the term commensal to refer to an animal that takes advantage of exploiting the resources of others (the hosts), while the animal's activity stays neutral to the host (9, 10).

It is widely accepted that interactions between cats and humans started as a commensal relationship (1, 11, 12). Synanthropization of the wildcat developed from occasional commensalism, initiated by availability of synanthropic rodents in agricultural landscapes. Through times, it led to nearly full dependency on anthropogenic resources and behavioral adaptation to the artificial environment and the proximity of humans, and eventually to mutualism which culminated in domestication. However, due to cats' solitary and territorial behavior, even modern domestic cats live somewhere along a continuum from close relationships with people to feral (13, 14). The cat's way to domestication is a complex and still unresolved topic with many questions concerning the chronology

Significance

Most of today's domesticates began as farm animals, but cat domestication took a different path. Cats became commensal of humans somewhere in the Fertile Crescent, attracted to early farmers' settlements by rodent pests. Cat remains from Poland dated to 4,200 to 2,300 y BCE are currently the earliest evidence for the migration of the Near Eastern wildcat to Central Europe. Tracking the possible synanthropic origin of that migration, we used stable isotopes to investigate the paleodiet. We found that the ecological balance was already changed due to the expansion of Neolithic farmlands. We conclude that among the Late Neolithic Near Eastern wildcats from Poland were free-living individuals, who preyed on rodent pests and shared ecological niches with native European wildcats.

Author contributions: M.K. and M.T.K. designed research; M.K., M.T.K., M.B., C.B., W.V.N., D.P., M.S.-P., B.W., J.W., M.W., and H.B. performed research; M.K., M.T.K., M.B., C.B., D.P., and H.B. analyzed data; M.K. and M.T.K. wrote the paper; M.B., C.B., W.V.N., D.P., M.S.-P., B.W., J.W., M.W., and H.B. reviewed the manuscript; and M.S.-P., J.W., and M.W. provided material/context.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

Data deposition: DNA sequences obtained in this study were deposited in GenBank under accession numbers MN537980 and MN537982.

[1]To whom correspondence may be addressed. Email: magkrajcarz@umk.pl.

This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1918884117/-/DCSupplemental.

of its dispersal with agricultural societies and the nature of its evolving relationship with humans.

The Near Eastern wildcat *Felis silvestris lybica* is the only subspecies of wildcat that has been domesticated (15). It is native to Northern Africa and the Near East. This subspecies is the ancestor of all modern domestic cats, *Felis silvestris catus*. Both wild and domesticated forms are very close genetically and cannot be discriminated with mitochondrial DNA (mtDNA) analysis (15). Therefore, in this paper, we regard them as one taxon, the Near Eastern cat (NE cat), or *F. s. lybica*/*catus*.

Interactions between humans and NE cats are believed to have begun in the Levant region around 7,500 to 7,200 y BCE (16) (*SI Appendix, Section 1*). The first known appearance of NE cat in the northern part of Europe (outside of the Mediterranean–Black Sea region) occurred in Poland about 3,600 to 2,300 y BCE (during the Late Neolithic Period) (17). The reason why the NE cat appeared so far from its native range is still not clear. Baca et al. (17) hypothesized that Late Neolithic NE cats in Poland, either still wild or already domesticated, followed the expansion northward of Neolithic farmers as their commensals. The geographic expansion of the NE cat was likely triggered by transformations of the landscape by Neolithic farmers, notably via deforestation (which created open environments similar to habitats exploited by the NE cat in its natural range) and the cultivation of crops, which increased the abundance of pest rodents (prey). It is noteworthy that NE cats spread into regions already occupied by the native European wildcat, *Felis silvestris silvestris*. The NE cat is genetically distinct from the European wildcat (15); even their fossils can be easily distinguished with mtDNA analysis (17, 18).

The understanding of the ecological and social status of the Late Neolithic NE cats in Poland is crucial to reconstruct the spatial and temporal history of human–cat interactions, which finally led to cat domestication and its current worldwide distribution. Therefore, in this study, we proposed to use stable isotopes to approximate the diet of Late Neolithic NE cats from Poland, which allowed us to identify possible synanthropic behaviors. By examining stable isotopic ratios in the remains of contemporary European wildcats and potential prey items, we explored the extent of niche partitioning between both felid species. We compared the results with the isotopic signature of pre-Neolithic and Early Neolithic European wildcats collected from the same region to explore the possible impact of the appearance of NE cats on the ecology of native European wildcats. We also compared the ecology of Late Neolithic NE cats with the earliest known domestic cats from Poland: i.e., from the Roman Period (19).

Neolithic Agricultural Landscape in Southern Poland

The earliest Neolithic settlements north of the Carpathian Mountains appeared about 5,500 y BCE (20). Fossils of the earliest NE cats collected in this part of Europe were dated to about 4,200 to 2,300 y BCE (*SI Appendix*, Tables S1 and S2), coinciding with a peak of Neolithic settlement density in the region that occurred between around 3,000 and 2,000 y BCE (21). Known in archaeology as the Late Neolithic Period, Eneolithic Period, or Second Phase of Neolithization, the interval includes a number of archaeological cultures, including the late phases of the Lengyel–Polgar Circle, Funnel Beaker Culture, Globular Amphorae Culture, Baden Culture, and Corded Ware Culture (22, 23). The largest archaeological settlement sites for these cultures were >50 ha in area (24), which suggests that some of them supported quite large human populations. Such high population densities must have led to extensive deforestation around the sites, especially because of slash and burn practices. Cultivated fields were probably rotated to maintain soil fertility, which further prevented the regeneration of forests (24, 25).

NE Cat Fossils and Site Contexts

We collected six remains of Late Neolithic NE cats from four cave sites in Kraków–Częstochowa Upland, southern Poland (17) (Fig. 1 and *SI Appendix, Section 2* and Table S1), which is situated close to the major settlement of the Funnel Beaker and Baden cultures from the neighboring Nida Basin region (23, 26). These settlements, as well as most of the other Late Neolithic sites known in southern Poland, were located on fertile, loess soils (26). The largest known site is Bronocice (24, 27), situated ∼45 km away from the NE cat-bearing caves. Other large sites (25, 26, 28) are situated about 30 km away. The Kraków–Częstochowa Upland was probably less intensely settled than the Nida Basin due to its rough and hilly landscape and rocky soils. The Neolithic inhabitants of the Upland are mostly known for their exploitation and processing of flint (29–31). In fact,

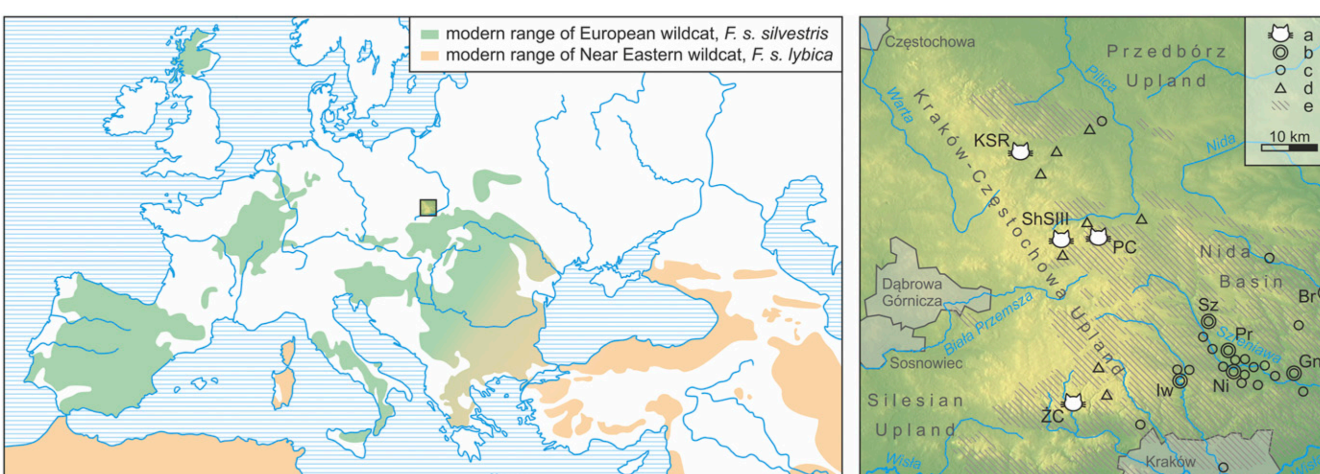

Fig. 1. Location of Central European sites with the Late Neolithic remains of NE cats (modern range of wildcat taxa after Ottoni et al.) (18): a, cave sites with NE cat remains (Krucza Skała Rockshelter [KSR], Perspektywiczna Cave [PC], Shelter in Smoleń III [ShSIII], and Żarska Cave [ŻC]); b, the largest Late Neolithic settlement sites in the region (Bronocice [Br], Gniazdowice [Gn], Iwanowice [Iw], Niedźwiedź [Ni], Prandocin [Pr], and Szczepanowice [Sz]) (26, 28); c, other well-documented, Late Neolithic settlement sites (26, 27, 49, 85, 86) (the state of archaeological recognition of the western and northern parts of the area is weak); d, Neolithic flint exploitation and/or flint processing site complexes (30, 31, 86); and e, loess cover (87).

workshops and mines occur within 5 km of some of the studied sites (Fig. 1).

Five of the six Late Neolithic NE cat remains were found in natural strata without any evidence of anthropogenic deposition. The only exception was a specimen from Żarska Cave, which was found within a layer of the Baden Culture. This specimen exhibited bite marks from a carnivore, suggesting that it was deposited there by a dog or another predator/scavenger. The other cat-bearing sites also yielded Late Neolithic archaeological material, but not directly connected with the NE cat fossils. At the Shelter in Smoleń III cave site, the remains of a child and dog were found, possibly representing a burial site (*SI Appendix, Section 2*). Moreover, charcoals found at the Perspektywiczna Cave and a fireplace and human bones excavated nearby at the Shelter in the Udorka Valley I are all dated to the Neolithic Period (*SI Appendix, Section 2*). These finds testify to the importance of the Kraków–Częstochowa Upland region to Neolithic societies and its close proximity to settlement centers.

The precise role of the NE cats in Late Neolithic agricultural societies is uncertain. No felid remains are known from the settlement sites. All remains have been found in caves where they could have been deposited by either natural or anthropic agents. Cave environments provide suitable conditions for bone preservation and favor the accumulation by bone collectors (predators and scavengers), who may explore both natural habitats and human settlements (32, 33). Therefore, bones of NE cats excavated from caves could represent either victims of other carnivores or cats that lived and died inside the caves. However, we also cannot rule out that cats were kept or hunted by humans who occasionally visited those caves. Cave deposits are often palimpsests of human and animal activities so all of the above-described scenarios are plausible.

Searching for Synanthropic Behavior in Fossil Records

The cat's synanthropic behavior, particularly its exploitation of synanthropic rodents as a source of easily accessible food, is thought to be responsible for its domestication (11, 16). This seems to be a very probable explanation because many of today's carnivores, such as red fox, stone marten, Eurasian badger, or raccoon, easily switch to synanthropic behaviors, especially in areas densely populated by humans (2, 5). One aspect of synanthropic behavior can be traced relatively easily in fossil specimens, which is diet. When agricultural landscapes emerged during the Neolithic Period, this new artificial environment provided new habitats, new ecological niches, and new types of food resources for animals. In particular, the cultivation of crops produced a large amount of easily accessible food for herbivores and omnivores (i.e., cereal grains and other cultivated plants). Stored food likely attracted pests that fed on crops, such as rodents. The relatively open agricultural landscape and its synanthropic rodents provided prey for many predators, which likely shifted their hunting preferences toward more easily accessible pest species and, in so doing, developed a commensal relationship with Neolithic farmers (1, 16).

Use of Stable Isotopes to Detect Ancient Diets

The main problem in characterizing the diet of a free-living animal is an animal's individualistic and temporal variability in its feeding preferences. In modern animals, feeding preferences can be identified by examining food left in stomachs or scats (14). However, the feeding habits of fossil animals are difficult to characterize because conventional techniques of dietary analyses cannot be applied. In contrast, the stable isotope analysis method has become an essential tool for investigating dietary preferences and the trophic paleoecology of past animals (34–36). The great advantage of analyzing stable isotopes is that it allows estimating the average diet of an individual during a long interval of its lifespan, including even several years (37).

The most useful tool in paleodietary studies is the analysis of stable isotopes of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$, respectively) (34, 38–40). Both elements are taken in by animals through their diets and are components of bone collagen, an animal tissue that can survive burial and fossilization (41). In the case of animals that rely on a high-protein diet, such as felids, the isotopic composition of bone collagen carbon and nitrogen primarily reflects those of the protein portion of the diet while some amount of carbon may also come from lipids and carbohydrates (42, 43) (*SI Appendix, Section 3*).

Modifications of $\delta^{15}N$ and $\delta^{13}C$ Values in Neolithic Agricultural Ecosystems

Several factors related to anthropic agricultural activities are known to change the isotopic signal of the environment, which is then reflected in the tissues of carnivores (such as felids) (Fig. 2). Farming practices can modify the nitrogen isotopic signature of cultivated plants (Fig. 2). Application of animal manure as a fertilizer causes an increase of $\delta^{15}N$ values in crops, even by several per mill, especially in cereals grains (44, 45). The elevated $\delta^{15}N$ signal often found in bones of Neolithic humans is thought to be due to a diet relying on intensively manured cereals (46, 47). We expect a similar effect in all synanthropic animals foraging on plants from manured fields, including domesticated ungulates fed with grains or straw (47) or grazing in manured pastures, in dogs eating similar foods as their owners (48), and in rodent pests foraging on the crop grains. Manuring of fields by Late Neolithic farmers has been identified at several sites (49, 50) situated about 20 to 65 km from sites with remains of Late Neolithic NE cats. Elevated isotopic values found there in emmer and einkorn grains (from 5.7 up to 7.6‰) testify to the manuring practices in the vicinity of the studied area, which likely also affected $\delta^{15}N$ signatures in local populations of herbivores and their predators.

Anthropogenic shift in $\delta^{13}C$ values in early agricultural ecosystems is more difficult to detect (Fig. 2 and *SI Appendix, Section 3*). This is because different types of anthropogenic activity had opposite effects on the isotopic composition of plants: Reduced canopy cover elevated the $\delta^{13}C$ (40, 51–54) while irrigation decreased the $\delta^{13}C$ values of cultivated plants (55, 56). Moreover, the isotopically distinct C_4 plants, nonnative to Central Europe, were relatively unimportant among cereals which were cultivated in the Neolithic Poland (20, 24). So an impact of Late Neolithic agriculture on $\delta^{13}C$ signature of ecosystems may be considered unreadable.

ANTHROPOLOGY

Results

Collagen Preservation. Collagen yields varied among samples (40.1 to 168.8 mg/g). All samples checked for C:N atomic ratios were in the range 2.9 to 3.5, which is within the acceptable range for fresh, uncontaminated, and unweathered collagen (41) (*SI Appendix*, Tables S2 and S3).

Stable Isotopes. The $\delta^{15}N$ of Late Neolithic NE cats ranged from 8.6‰ to 9.3‰ whereas $\delta^{13}C$ ranged from −20.0‰ to −19.0‰. Contemporary European wildcats showed wider ranges (from 8.3‰ to 9.4‰ for $\delta^{15}N$ and from −20.1‰ to −18.4‰ for $\delta^{13}C$). Thus, the isotopic values of NE cats and contemporary European wildcats overlap (Fig. 3 and *SI Appendix*, Table S2). Pre-Neolithic–Early Neolithic European wildcats showed lower values both for $\delta^{15}N$ (from 7.3‰ to 8.3‰) and $\delta^{13}C$ (from −20.1‰ to −19.6‰). We found a significant statistical difference in $\delta^{15}N$ between three taxonomic/chronological felid groups (ANOVA: $F_{2,10} = 7.737$, $P = 0.0093$) (*SI Appendix*, Table S5). Late Neolithic NE cats were significantly different from pre-Neolithic–Early Neolithic European wildcats (Tukey's post hoc $P = 0.0076$), but not from the contemporary European wildcats (Tukey's post hoc $P = 0.6209$) (*SI Appendix*, Table S6). Late Neolithic and pre-Neolithic–Early Neolithic

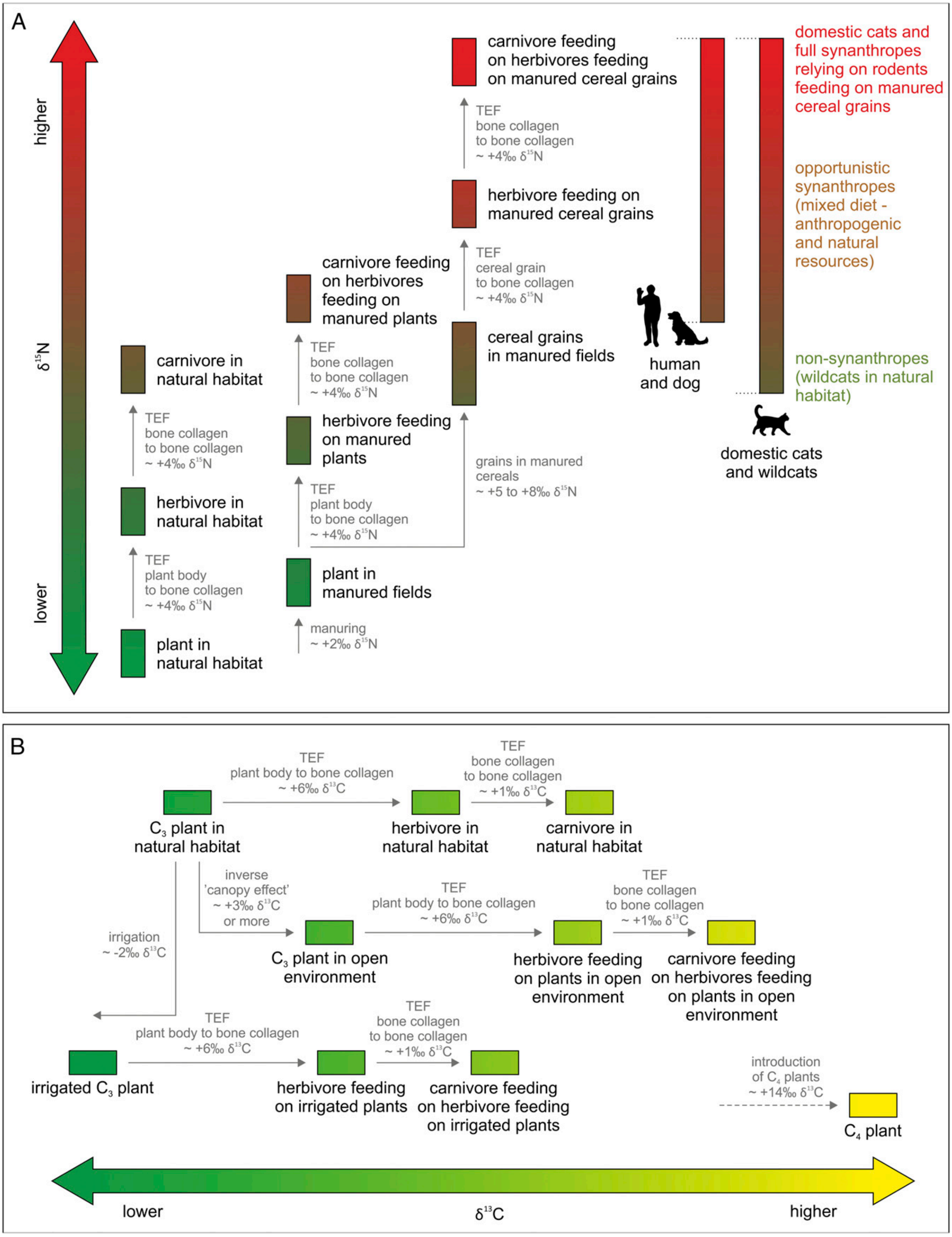

Fig. 2. Schematic depiction of modifications of the isotopic composition of plants, herbivores, and carnivores in an agricultural landscape. (*A*) Modifications of the $\delta^{15}N$ signal; theoretical values for human and dog include plant diet based on manured cereal grains, and meat of herbivores feeding on manured plants and cereal grains; felid signal ranges from diet of carnivores in natural habitats to carnivores feeding on herbivores (rodents) feeding on manured cereal grains. (*B*) Modifications of the $\delta^{13}C$ signal. Data for isotopic shifts obtained from literature (44, 45, 47, 51, 52, 75, 88). TEF, trophic enrichment factor.

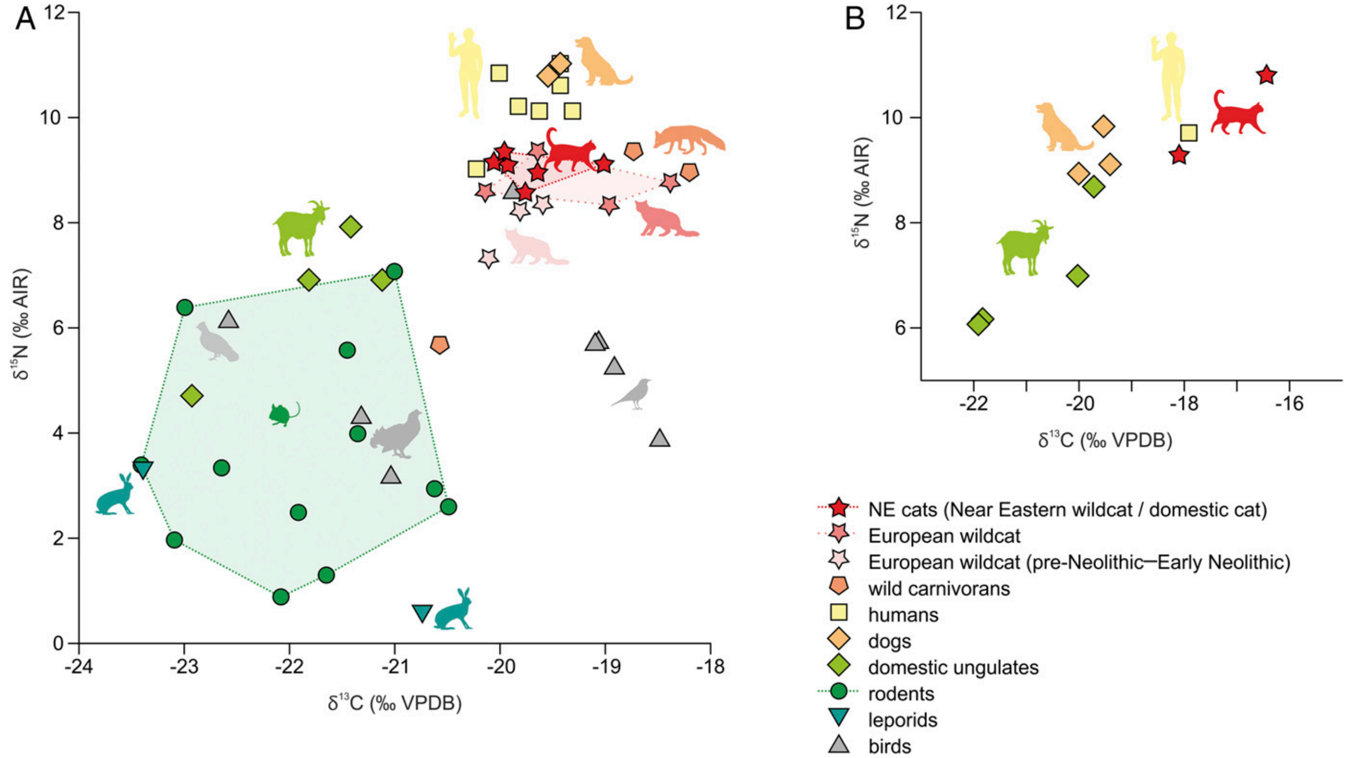

Fig. 3. Data for $\delta^{13}C$ and $\delta^{15}N$ values in bone collagen. (*A*) Late Neolithic animals and humans from Kraków–Częstochowa Upland (data for humans and domestic animals from literature are included) (64). (*B*) Roman Period animals and humans from Kuiavia (Northern Poland) (data for humans from literature) (89).

European wildcats were also significantly different in terms of $\delta^{15}N$ (Tukey's post hoc $P = 0.04498$). No significant difference in $\delta^{13}C$ was found between any felid groups (ANOVA: $F_{2,10} = 1.392$, $P = 0.2928$) (*SI Appendix*, Table S5).

Humans and domestic dogs seemed to be very close isotopically to each other and showed the highest $\delta^{15}N$ among all analyzed samples. Wild birds and herbivorous/omnivorous mammals (domestic mammals, rodents, and leporids) showed high variability in $\delta^{15}N$ and $\delta^{13}C$ signals (Fig. 3 and *SI Appendix*, Table S2). Among the suite of species we analyzed, we assumed that birds, rodents, and leporids were potential prey of felids (both NE cats and European wildcats), which is in agreement with known dietary habits of modern felids (57–59). Because these three taxonomic groups of prey overlapped in $\delta^{15}N$ and $\delta^{13}C$ values, we applied cluster analysis to subdivide prey on the basis of isotopic results. This analysis revealed three clusters, representing three different isotopic and ecological groups, named hereafter as clusters A, B, and C (Fig. 4). We interpreted these clusters as wild forest herbivores/omnivores with low $\delta^{15}N$ (cluster A), synanthropic herbivores/omnivores foraging in agricultural areas with high $\delta^{15}N$ (cluster B), and wild omnivorous migratory birds (cluster C) (*SI Appendix*, *Section 4*). We found significant difference in $\delta^{15}N$ and

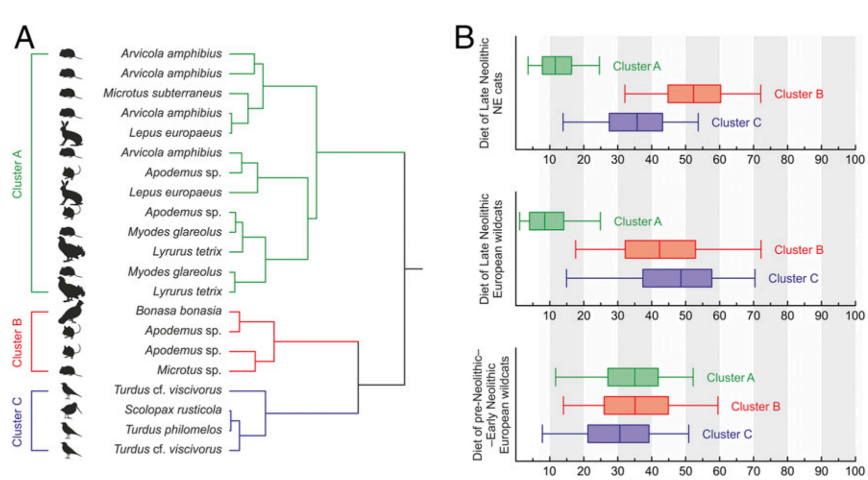

Fig. 4. Isotopic groups of prey in the diet of Late Neolithic and pre-Neolithic–Early Neolithic felids. (*A*) groups of prey revealed by cluster analysis. (*B*) Proportions of these prey groups in the diet of the studied felids based on MixSIAR reconstruction.

$\delta^{13}C$ between these clusters (ANOVA for $\delta^{15}N$: $F_{2,18} = 22.81$, $P < 10^{-4}$; Welch test for $\delta^{13}C$: $F_{7,213} = 52.02$, $P < 10^{-4}$) (*SI Appendix*, Table S5).

Diet Reconstruction. We estimated the proportion of three prey clusters in the diet of NE cats, contemporary European wildcats, and pre-Neolithic–Early Neolithic European wildcats, using the Bayesian mixing model (MixSIAR). The model showed convergence in two tests of diet reconstructions. Both the Gelman–Rubin and Geweke diagnostic tests examined 34 variables of the model. In the Gelman–Rubin test, no variable scored higher than 1.01 whereas, out of 34 variables, the Geweke test revealed no unequal variables in chain 1, no unequal variables in chain 2, and only one unequal variable in chain 3. Therefore, we assumed that the calculated model was perfectly applicable for reconstructing diets (Fig. 4 and *SI Appendix*, Table S7).

Discussion

Synanthropic Signal of NE Cat Diet during the Late Neolithic Period. The first isotopic report on commensal behavior of ancient wild felids examined Neolithic cats from China (60). Based on $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen, Hu et al. (60) identified a substantial consumption of millet-based food by humans, rodents, and cats, which suggested a possible commensal or even mutualistic behavior of Neolithic cats. However, lack of data for contemporary rodents and other possible prey limited the interpretative potential of those results (61). Furthermore, morphometric verification of Chinese cat remains revealed that the study involved a leopard cat (*Prionailurus bengalensis*) rather than a wildcat or domestic cat (62). Despite no direct relation to *Felis*, that study pioneered the field of ancient felid ecology.

In our dataset of Central European Late Neolithic ecosystem, the $\delta^{15}N$ values of felids were all lower than they were for contemporary humans and dogs. This result would appear to contradict an assumption that top predators must exhibit the highest isotopic signature in their ecosystem (36). This is, however, true in the case of a single food web. In more complex ecosystems, the particular food webs which rely on different resources may exhibit different isotopic signatures of equivalent trophic levels (63). Our result is in line with the isotopic relationships observed in modern ecosystems that include agricultural biotopes (4). That is, if manuring is taken into consideration in predicting $\delta^{15}N$ values, animals which regularly consume cereal grain-rich food may eventually exhibit higher $\delta^{15}N$ than animals relying on a pure meat (protein-rich) diet (Fig. 2). Our data revealed the highest $\delta^{15}N$ in humans and dogs, which suggests they consumed a diet rich in cereal grains (such as flour, bread, pearl barley, etc.). In addition, isotopic data for humans, dogs, and livestock known from other Neolithic sites in southern Poland show similar trends of elevated $\delta^{15}N$ (64).

Late Neolithic NE cats showed lower $\delta^{15}N$ than did humans and dogs. That suggests they were not fully dependent on human food supplies and instead also exploited other food sources available in their habitats. In general, felids are obligate carnivores that require a diet consisting primarily of meat. Cats are excellent hunters; even housecats can easily become feral and survive in the wild. In fact, some domestic cats temporarily abandon human owners and live for a time without human attention (65, 66). Our results indicated that NE cats fed independently from humans, which suggests they were still wild or feralized or people did not pay attention to their feeding.

Stable isotopes indicated that the most important component of NE cats' diet were animals with relatively high $\delta^{15}N$ values, such as mice, voles, and hazel grouse (cluster B). We interpret this prey as synanthropes feeding on plants with elevated $\delta^{15}N$ due to agricultural activity (manuring). The synanthropic rodents, however, were not as high in $\delta^{15}N$ as some domestic animals and humans (Fig. 3). This main component of diet was followed by wild omnivorous migratory birds, such as thrushes and woodcocks (cluster C). A minor part of their diet (5 to 25%) was constituted of wild forest animals (cluster A). The high proportion of prey from cluster B is especially relevant because this group likely represents pests of farmed crops. These NE cats certainly lived in a human-modified environment and were involved in the synanthropic food web.

Drawing a broader picture of relationships between the NE cats and Neolithic people is limited by taphonomic factors and depositional contexts of the sampled remains. Firstly, the number of yet discovered remains is low. With only several specimens in hand, we have to consider that our results may represent individual-related behaviors rather than population-scale trends. Secondly, the available NE cat remains come from non-anthropogenic contexts, namely caves situated at some distance from farming settlements (Fig. 1). Such remains may represent feralized individuals, who stepped away from closer relationships with humans and started to live on their own, somewhere between the natural and agricultural landscapes. This scenario seems likely when comparing the stable isotope signature of the studied NE cats with those of Roman Period domestic cats from Poland. Remains of those Roman Period cats were found at settlement sites (19) so their isotopic signature may be representative for individuals living in farmland. The stable isotopes of cats from the Roman Period were closer to those of humans and dogs, which means that their food was more similar to the basic diet of humans and dogs during this period (Fig. 3 and *SI Appendix*, Tables S2 and S3). Their high $\delta^{13}C$ values may be presumably linked to advanced deforestation and/or widespread cultivation of C_4 plants, but their human-like $\delta^{15}N$ signal suggests that either these cats were fed by their human owners, or that rodent pests caught by the cats close to the farms bore a stronger synanthropic signal. We may assume that Late Neolithic cats who lived closer to the human settlements (or within settlements) than the studied specimens might bear an isotopic signal similar to that of the Roman Period cats.

In another hypothetical scenario, consistent with the interpretation of Baca et al. (17), the Late Neolithic NE cats from Poland were still-wild animals who followed the Neolithic farms in search for easily available prey. Isotopic results cannot answer whether the Late Neolithic NE cats migrated to Central Europe as full-fledged domesticates or simply synanthropes until samples of NE cats from farming settlements are recovered. However, from the obtained isotopic data, we can extract the following observations, which help to reconstruct the ecology of the studied NE cats:

1) The Late Neolithic NE cats were clearly distinct in terms of stable isotope composition (and as a consequence, in terms of diet) from contemporary humans and dogs, and also from highly anthropic Roman Period cats who lived in farmland (Fig. 3).
2) The NE cats were isotopically different from pre-Neolithic–Early Neolithic European wildcats (statistically significant difference) (*SI Appendix*, Table S6), who certainly were nonsynanthropic, free-living felids.
3) The NE cats' diet included both synanthropic herbivores/omnivores foraging in agricultural areas (cluster B—prevailing in diet) and wild forest herbivores/omnivores (cluster A—minor part of diet) (Fig. 4 and *SI Appendix*, Table S7).

Based on the facts above, we can conclude that the studied NE cat individuals were opportunistic synanthropes, exploiting both anthropogenic and natural ecosystems. Their subsistence relied mostly on the agricultural landscape as synanthropic prey constituted a major part of their diet. At the same time, they were not dependent on food supplied by humans. Our results point toward their behavioral flexibility.

Changes in the Trophic Niche of European Wildcats during the Neolithic Period. When NE cats appeared in southern Poland during the Neolithic Period, the territory was occupied by a native European wildcats. The emergence of Neolithic agriculture was not without impact on their ecology. Our results show a shift in the proportion of prey types consumed by European wildcats over time from the pre-Neolithic–Early Neolithic Period to the Late Neolithic Period (Fig. 4 and *SI Appendix*, Table S7), supported by statistical tests (Tukey's post hoc for $\delta^{15}N$ $P = 0.04498$) (*SI Appendix*, Table S6). The most apparent change was the reduction in participation of wild forest herbivores/omnivores (cluster A) in diet from about 30 to 40% in the pre-Neolithic–Early Neolithic Period to less than 20% by the Late Neolithic Period. This reduction in the proportion of cluster A in diet was accompanied by an increase in prey from clusters B (synanthropic rodents) and C (wild omnivorous migratory birds). This shift may reflect an increased predation of synanthropic rodent and thrush populations when open environments expanded in the landscape. Possible scenarios responsible for this shift include a change of European wildcat's trophic niche (being either an effect of the loss of prey due to anthropogenic pressure or adaptation toward hunting for more abundant and available prey) or a change in the food base of their prey.

Coexistence of NE Cats and European Wildcats. During the Late Neolithic Period, the European wildcat and NE cat coexisted in southern Poland. European wildcat and domestic cat are closely related and exhibit a similar prey choice (65). Where they occupied the same territory, it can be expected that some level of competition would occur. Our results indicate that both studied felids shared an ecological niche, as can be inferred from the lack of significant statistical difference between the isotopic composition of both groups (*SI Appendix*, Table S6). In addition, over 99% of the standard ellipse area (SEAc—corrected for the small sample size) of stable isotopes for Late Neolithic NE cats overlaps with the SEAc of the contemporary European wildcats (Table 1). This means that the Late Neolithic European wildcats equaled the studied NE cat specimens in level of synanthropy, and both taxa might be classified as opportunistic synanthropes.

These results raise important questions about the nature of the prehistoric coexistence of the two felids. Did they compete? If so, which one was a stronger competitor? Alternatively, did they partition the niche? The answers come from the diet reconstruction of both taxa (Table 1 and Fig. 4). The native European wildcat occupied much wider isotopic niches than the NE cat's niche (i.e., only about 21% of European wildcat SEAc overlaps with values for the NE cat). The native subspecies was more oriented toward the prey of cluster C (wild omnivorous migratory birds) whereas the NE cat seems to have been more oriented toward the prey of cluster B (synanthropic rodents). These dissimilarities may represent an attempt to avoid competition or may signify different behaviors of the two taxa. We can reasonably assume that trophic interactions between Late Neolithic populations were similar to interactions between today's European wildcats and feral domestic cats (13, 65). The recent feral domestic cats focus on synanthropic prey items while wildcats living in the same region tend to avoid open areas and prey on larger or arboreal forest animals (14). The studied Late Neolithic NE cat specimens could be classified among the casual synanthropes while the Late Neolithic European wildcats were probably rather tangential synanthropes (*SI Appendix*, Fig. S1).

However, after the appearance of the NE cat, the native European wildcat did not avoid the ecological niche occupied by the newcomer, but it instead expanded to a similar niche, as discussed above. This shift indicates that the NE cat was not a serious competitor with the native European wildcat, and/or the new anthropogenic habitat was broad enough to be exploited by both felid species simultaneously. According to one of the hypothetical scenarios where the studied specimens were feralized individuals, the impact of NE cat population on the native wildcat was limited by the number of feral runaways. In the alternative scenario assuming that NE cats were still-wild synanthropes following the farmlands, our specimens may represent the part of the population which was more oriented toward exploiting forest resources. However, each scenario implies that NE cats coexisted and shared the ecological niches with native European wildcats as early as the Neolithic Period.

Table 1. Overlapping of the Standard Ellipse Areas corrected for small sample size (SEAc) for isotopic values of taxonomic/chronological groups of felids

	% of SEAc area covered by SEAc of:		
SEAc	Late Neolithic NE cat	Late Neolithic European wildcat	Pre-Neolithic–Early Neolithic European wildcat
Late Neolithic NE cat		99.2	0.0
Late Neolithic European wildcat	21.1		3.2
Pre-Neolithic–Early Neolithic European wildcat	0.0	19.0	

Conclusions

One of the key issues for understanding the process of domestication is to determine the type of ecological relationships that existed between humans and a given species. Among the wild animals which have been domesticated, the cat ancestors were unique due to their solitary, territorial behaviors. The cat's domestication was a complex process with many questions remaining concerning the history of the cat's relationship with humans and its patterns of dispersal worldwide.

The early appearance of the ancestors of modern domestic cats in Late Neolithic Poland, far from the native range, suggests a migration from the Near East with early farmers and a synanthropic behavior. In our study, we reconstructed the diet of these cats using stable isotope methods, to track their role in Neolithic agricultural ecosystems.

We found that the isotopic signature of Late Neolithic ecosystems was highly variable, likely due to the close cooccurrence of natural ecosystems and grain crop agriculture. Humans, dogs, and domestic farm animals from that period show expectedly high $\delta^{15}N$ values. A moderately elevated $\delta^{15}N$ signal also occurred in some rodents, likely because the pests consumed grain crops grown by people. The isotopic signature of Late Neolithic NE cats suggests that they were free-living, not dependent on a human-produced food, and preyed upon synanthropic mice and voles (i.e., crop pests). The NE cats shared their isotopic niche with European wildcats although the native subspecies utilized a much broader niche than the NE cats did. The coexistence and niche sharing likely induced some level of competition and created an opportunity to hybridize between the two taxa. This provides serious implications for the history of wildcat gene pool contamination by NE/domestic cats and for the conservation of this species. However, the full understanding of this past hybridization requires further nuclear DNA studies of fossil specimens.

How close the relationship was between Late Neolithic NE cats and humans that once inhabited present-day Poland, and whether those cats were already domesticated, is still an open question. Searching for cat remains among archaeozoological material from Neolithic settlement sites may provide an insight into the human/cat relationship. Moreover, to obtain a comprehensive history of cat domestication and its dispersal, additional well-dated remains from other regions of Europe are needed.

Materials and Methods

Sampled Material. In this study, we presented results of isotopic analyses of Late Neolithic *F. s. lybica/catus* and *F. s. silvestris* fossil remains, published in our previous study (17). We sampled exactly the same specimens, genetically identified by Baca et al. (17). Only the specimen from Krucza Skała Rock-shelter was not included here because it had been entirely consumed by previous analyses. Since our previous publication, we had identified two more specimens of *F. s. lybica/catus* and three of *F. s. silvestris*, which we also included in our analyses (*SI Appendix*, Fig. S2 and Tables S1, S2, and S8). Altogether, we present data for six NE cats and four European wildcats of similar chronology and from the same study area (Central Europe). In addition, we examined three specimens of the European wildcat of pre-Neolithic and Early Neolithic age, and two NE cats known from northern Poland and dated to the Roman Period (19). Our material includes also remains of other animals and humans of the same chronology as the studied felids and found in the same area, to provide wider ecological context. We confirmed the geological ages of all studied felid specimens with direct radiocarbon dating and tested their taxonomy by analyzing ancient mtDNA.

Collagen Stable Isotopes Analysis. We performed stable isotope analysis of carbon and nitrogen on bone collagen of sampled specimens. We first cleaned small bone fragments by rinsing them with acetone and distilled water and then dried them and crushed them to a powder of <0.7 mm grain size. We used ~0.05 to 0.5 g of bone powder for collagen extraction. In case of rodents whose identifiable bones were too small, we joined several specimens of the same taxon and the same stratigraphy together into one sample in order to collect sufficient weight (samples nos. CAT 9, CAT 27, CAT 44, CAT 45, CAT 46, CAT 47, and CAT 53) (*SI Appendix*, Table S2). We purified the collagen according to a well-established protocol (67). We performed all elemental and isotopic measurements at the Stable Isotopes Laboratory at the Institute of Geological Sciences, Polish Academy of Sciences (Warszawa, Poland) using a Flash EA 1112HT elemental analyzer (Thermo Scientific) connected to a Delta V Advantage mass spectrometer (Thermo Scientific). Mean SEs were <0.33‰ for $\delta^{13}C$ and <0.43‰ for $\delta^{15}N$.

We expressed isotopic values as δ (isotopic ratio over the ratio of an appropriate standard) in parts per million (‰), as follows: $\delta^{E}X = (R_{sample}/R_{standard} - 1) \times 1{,}000$, where ^{E}X is ^{13}C or ^{15}N and $R = {}^{13}C/{}^{12}C$ (or $^{15}N/^{14}N$). The international references were Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric nitrogen (AIR) for nitrogen. We normalized all measurements to $\delta^{13}C$ values of USGS40 and USGS41 standards and all $\delta^{15}N$ values to IAEA 600 standard.

We checked the quality of extracted collagen using their chemical compositions (%C, %N, and C:N ratios) (*SI Appendix*, Tables S2 and S3) using a Vario EL III elemental analyzer with sulfanilic acid as an internal standard. To be acceptable for further analysis, expected values of well-preserved collagen must be similar to values of collagen extracted from fresh bones (41, 68). Samples with C:N ratios in the range of 2.9 to 3.6 were accepted.

Diet Reconstruction and Statistics. To reconstruct the diets of felids, it was necessary to attribute each potential prey sample to groups with clearly recognizable isotopic differences. If we would use every species as its own group, the overlapping zones among prey species would have been too large to differentiate samples, and the statistics program MixSIAR (see below) would not have worked effectively. We were able to partition prey samples into three distinct groups, based on a cluster analysis using JMP 14 (69).

We used MixSIAR (Bayesian Mixing Models in R) (70), widely applied in ecological and archaeological studies (e.g., refs. 71 and 72), to reconstruct the protein fraction of a felid's diet based on the proportion of prey. We followed the methodology presented by Baumann et al. (73). The Bayesian statistical calculations of this package are quite robust for small sample sizes ($n < 20$) (74). MixSIAR allowed us to reconstruct the most likely diet of the sampled felids based on the differences in nitrogen and carbon isotope values between their bone collagen and the bone collagen of their potential prey. We identified prey resources as clusters A, B, and C. We used trophic enrichment factor (TEF) values ($\Delta^{13}C = 1.1 \pm 1.1$‰ and $\Delta^{15}N = 3.2 \pm 1.8$‰) from a study of modern foxes (75). TEF values reflect the enrichment in heavy nitrogen and heavy carbon isotopes in predator bone collagen in relation to the bone collagen of its prey and therefore reflect the behavior and physiology of the analyzed consumers (71, 75, 76). To get a robust statistical analysis, we set the Markov chain Monte Carlo (MCMC) chain length to 1,000,000 with a burn-in of 500,000 in three chains (70, 73). Gelman–Rubin and Geweke tests were applied to check the model's convergence. The perfect convergence is showed by Gelman–Rubin test's value near 1.0; however, values below 1.1 are acceptable (77). According to the Geweke test, the model is convergent if the means of the first and the second part of each chain, using a two-sided z-test, are the same (70, 73).

To examine the trophic niches of the three felid groups (pre-Neolithic–Early Neolithic *F. s. silvestris*, Late Neolithic *F. s. silvestris*, and Late Neolithic *F. s. lybica/catus*), we used the R package Stable Isotope Bayesian Ellipses in R (SIBER), following the protocol of Jackson et al. (78). To determine the breadth of the niches, we calculated the standard ellipse area (SEA) and the standard ellipse area corrected for sample size (SEAc), using most likelihood estimates. Because the most likelihood estimate explains 40% of data, it is recommended to calculate core niche (78). This core niche estimate is still informative even with smaller sample sizes.

To check for statistical differences between the three felid groups as well as the prey clusters, we used an ANOVA test or, alternatively, Welch *F* and Kruskal–Wallis tests in the case of unequal variance. The homogeneity of variance was tested with Levene's test, and normality of the tested sample sets with the Shapiro–Wilk test. In the case of significant ANOVA or Kruskal–Wallis tests, we applied post hoc pairwise comparisons (Tukey's test or Mann–Whitney *U* test and Dunn's tests, respectively), with Bonferroni correction of *P* values for multiple comparisons, due to low sample sizes. We used PAST software, Ver. 3.26 (79), for analysis of variance and pairwise comparisons.

Analysis of Ancient DNA. For the specimens found after the publication of Baca et al. (17), we performed DNA analyses following the same methodology. First, we extracted DNA from five new specimens (CAT 11, CAT 16, CAT 50, CAT 155, and CAT 157) following the procedure described by Dabney et al. (80). We directly converted genomic DNA into double-indexed sequencing libraries following Kircher et al. (81), with minor modifications (82). Target enrichment of mtDNA, sequencing on Illumina platform, and processing of the sequence reads were performed as in Baca et al. (17). For two samples (CAT 11, CAT 157), which we found carrying *F. s. lybica/F. s. catus* mtDNA, we conducted phylogenetic analyses to determine their mtDNA lineages. We reconstructed phylogeny, based on a 2,604-base pair (bp) fragment of mtDNA, using Bayesian and maximum likelihood (ML) approaches with MrBayes 3.2.6 and PhyML 3.1, respectively (*SI Appendix*, Fig. S2). We used the partitioning scheme and substitution models used by Baca et al. (17). The Bayesian analysis consisted of two independent runs with four coupled chains, each run for 10 million MCMC generations with trees sampled every 1,000th generation. In the ML analysis, we chose the best tree from those obtained using the subtree pruning and regrafting (SPR) and nearest-neighbor interchange (NNI) tree-searching algorithms. We assessed branch support using an approximate likelihood-ratio test (aLRT) using the Shimodaira–Hasegawa aLRT (SH-aLRT) procedure.

Radiocarbon Dating. We performed radiocarbon dating directly on sampled bones (*SI Appendix*, Table S1) by analyzing the bones in the Radiocarbon Laboratory in Poznań, Poland, using the accelerator mass spectrometry (AMS) method for extracted collagen. We calibrated the obtained ^{14}C ages with the IntCal'13 radiocarbon calibration curve (83), using OxCal Ver. 4.2.4 software (84).

ACKNOWLEDGMENTS. We thank Daniel Makowiecki for his assistance and constructive comments given during preparation of the manuscript and Teresa Tomek for providing taxonomical identification of bird bones. The study was supported by National Science Centre, Poland Grant 2017/27/B/NZ8/00728. The excavations at Shelter in Smoleń III, Perspektywiczna Cave, and Żarska Cave were supported by National Science Centre, Poland Grants 2011/01/N/HS3/01299, 2014/15/D/HS3/01302, and 2013/11/D/HS3/01877, respectively. Sequencing was performed thanks to Next Generation Sequencing Core Facility CeNT UW, using NextSeq 500 platform. We thank the editor and three anonymous reviewers for constructive comments and criticism that helped us to improve the manuscript.

1. M. A. Zeder, The domestication of animals. *J. Anthropol. Res.* **68**, 161–190 (2012).
2. J. D. Vigne, The origins of animal domestication and husbandry: A major change in the history of humanity and the biosphere. *C. R. Biol.* **334**, 171–181 (2011).
3. N. Russell, The wild side of animal domestication. *Soc. Anim.* **10**, 285–302 (2002).
4. M. Magioli *et al*., Human-modified landscapes alter mammal resource and habitat use and trophic structure. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 18466–18472 (2019).
5. P. W. Bateman, P. A. Fleming, Big city life: Carnivores in urban environments. *J. Zool. (Lond.)* **287**, 1–23 (2012).

6. T. O'Connor, *Animals As Neighbors: The Past and Present of Commensal Animals*, (Michigan State University Press, 2013).
7. R. F. Johnston, "Synanthropic birds of North America" in *Avian Ecology in An Urbanizing World*, J. M. Marzluff, R. Bowman, R. Donnelly, Eds. (Springer Science+Business Media, LLC, 2001), pp. 49–67.
8. M. L. McKinney, Urbanization as a major cause of biotic homogenization. *Biol. Conserv.* **127**, 247–260 (2006).
9. C. R. Dickman, Commensal and mutualistic interactions among terrestrial vertebrates. *Trends Ecol. Evol. (Amst.)* **7**, 194–197 (1992).
10. S. E. Jorgensen, B. D. Fath, Eds., *Encyclopedia of Ecology*, (Elsevier B.V., ed. 1, 2008).
11. C. A. Driscoll, D. W. Macdonald, S. J. O'Brien, From wild animals to domestic pets, an evolutionary view of domestication. *Proc. Natl. Acad. Sci. U.S.A.* **106** (suppl. 1), 9971–9978 (2009).
12. G. Larson, D. Q. Fuller, The evolution of animal domestication. *Annu. Rev. Ecol. Evol. Syst.* **45**, 115–136 (2014).
13. Z. Biró, L. Szemethy, M. Heltai, Home range sizes of wildcats (*Felis silvestris*) and feral domestic cats (*Felis silvestris* f. *catus*) in a hilly region of Hungary. *Mamm. Biol.* **69**, 302–310 (2004).
14. Z. Biró, J. Lanszki, L. Szemethy, M. Heltai, E. Randi, Feeding habits of feral domestic cats (*Felis catus*), wild cats (*Felis silvestris*) and their hybrids: Trophic niche overlap among cat groups in Hungary. *J. Zool. (Lond.)* **266**, 187–196 (2005).
15. C. A. Driscoll *et al*., The Near Eastern origin of cat domestication. *Science* **317**, 519–523 (2007).
16. J. D. Vigne, J. Guilaine, K. Debue, L. Haye, P. Gérard, Early taming of the cat in Cyprus. *Science* **304**, 259 (2004).
17. M. Baca *et al*., Human-mediated dispersal of cats in the Neolithic Central Europe. *Heredity* **121**, 557–563 (2018).
18. C. Ottoni *et al*., The palaeogenetics of cat dispersal in the ancient world. *Nat. Ecol. Evol.* **1**, 139 (2017).
19. M. Krajcarz *et al*., On the trail of the oldest domestic cat in Poland. An insight from morphometry, ancient DNA and radiocarbon dating. *Int. J. Osteoarchaeol.* **26**, 912–919 (2016).
20. A. Czekaj-Zastawny, "The first farmers from the south–Linear Pottery culture" in *The Past Societies. Polish Lands from the First Evidence of Human Presence to the Early Middle Ages 2: 5500-2000 BC*, P. Włodarczak, Ed. (Institute of Archaeology and Ethnology, Polish Academy of Sciences, 2017), pp. 20–62.
21. A. Timpson *et al*., Reconstructing regional population fluctuations in the European Neolithic using radiocarbon dates: A new case-study using an improved method. *J. Archaeol. Sci.* **52**, 549–557 (2014).
22. M. Nowak, The second phase of Neolithization in East-Central Europe. *Antiquity* **75**, 582–592 (2001).
23. P. Włodarczak, Ed., *The Past Societies. Polish lands from the First Evidence of Human Presence to the Early Middle Ages 2: 5500-2000 BC*, (Institute of Archaeology and Ethnology, Polish Academy of Sciences, 2017).
24. S. Milisauskas, J. Kruk, R. Ford, M. Lityńska-Zając, Neolithic plant exploitation at Bronocice. *Spraw. Archeol.* **64**, 77–112 (2012).
25. M. Moskal-del Hoyo *et al*., Open country species persisted in loess regions during the Atlantic and early Subboreal phases: New multidisciplinary data from southern Poland. *Rev. Palaeobot. Palynol.* **253**, 49–69 (2018).
26. J. Kruk, *Studia Osadnicze nad Neolitem Wyżyn Lessowych*, (Zakład Narodowy im. Ossolińskich, 1973).
27. J. Kruk, S. Alexandrowicz, S. Milisauskas, Z. Śnieszko, *Osadnictwo i Zmiany Środowiska Naturalnego Wyżyn Lessowych. Studium Archeologiczne i Paleogeograficzne nad Neolitem w Dorzeczu Nidzicy*, (Instytut Archeologii i Etnologii Polskiej Akademii Nauk, 1996).
28. S. Kadrow, "Miejsca szczególne w neolicie i w epoce brązu - Iwanowice, Gródek Nadbużny" in *Miejsca Pamięci. Pradzieje, Średniowiecze i Współczesność*, B. Gediga, A. Grossman, W. Piotrowski, Eds. (Instytut Archeologii i Etnologii PAN, Muzeum Archeologiczne w Biskupinie, 2015), pp. 201–216.
29. E. Rook, Osadnictwo neolityczne w jaskiniach Wyżyny Krakowsko-Częstochowskiej. *Mater. Archeol.* **20**, 5–130 (1980).
30. J. Lech, "Wczesny i środkowy neolit Jury Ojcowskiej" in *Jura Ojcowska w Pradziejach i w Początkach Państwa Polskiego*, J. Lech, J. Partyka, Eds. (Ojcowski Park Narodowy–Muzeum im. Prof. Władysława Szafera, 2006), pp. 387–438.
31. A. Pelisiak, The exploitation and distribution of flints from the central part of Polish Jura in the Late Neolithic times. *Analecta Archaeol. Ressoviensia* **1**, 73–86 (2006).
32. M. Krajcarz, M. T. Krajcarz, The red fox (*Vulpes vulpes*) as an accumulator of bones in cave-like environments. *Int. J. Osteoarchaeol.* **24**, 459–475 (2014).
33. P. Andrews, *Owls, Caves, and Fossils: Predation, Preservation, and Accumulation of Small Mammal Bones in Caves, with An Analysis of the Pleistocene Cave Faunas from Westbury-Sub-Mendip, Somerset, UK*, (University of Chicago Press, ed. 1, 1990).
34. P. L. Koch, "Isotopic study of the biology of modern and fossil vertebrates" in *Stable Isotopes in Ecology and Environmental Science*, R. Michener, K. Lajtha, Eds. (Blackwell Publishing Ltd, 2007), pp. 99–154.
35. H. Bocherens, D. Drucker, "Terrestrial teeth and bones" in *Encyclopedia of Quaternary Sciences*, S. Elias, Ed. (Elsevier, ed. 2, 2013), Vol. 1, pp. 304–314.
36. H. Bocherens, Isotopic tracking of large carnivore palaeoecology in the mammoth steppe. *Quat. Sci. Rev.* **117**, 42–71 (2015).
37. S. C. Münzel *et al*., Behavioural ecology of Late Pleistocene bears (*Ursus spelaeus, Ursus ingressus*): Insight from stable isotopes (C, N, O) and tooth microwear. *Quat. Int.* **339–340**, 148–163 (2014).
38. J. F. Kelly, Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Can. J. Zool.* **78**, 1–27 (2000).
39. H. Bocherens, "Preservation of isotopic signals (^{13}C, ^{15}N) in Pleistocene mammals" in *Biogeochemical Approaches to Paleodietary Analyses*, M. Katzenberg, S. Ambrose, Eds. (Kluwer Academic/Plenum Publishers, 2000), pp. 65–88.
40. H. Fricke, "Stable isotope geochemistry of bonebed fossils: Reconstructing paleoenvironments, paleoecology, and paleobiology" in *Bonebeds. Genesis, Analysis, and Paleobiological Significance*, R. R. Rogers, D. A. Eberth, A. R. Fiorillo, H. Fricke, Eds. (University of Chicago Press, 2008), pp. 437–490.
41. M. J. DeNiro, Postmortem preservation and alteration of in vivo bone collagen isotope ratios in relation to palaeodietary reconstruction. *Nature* **317**, 806–809 (1985).
42. S. Jim, V. Jones, S. H. Ambrose, R. P. Evershed, Quantifying dietary macronutrient sources of carbon for bone collagen biosynthesis using natural abundance stable carbon isotope analysis. *Br. J. Nutr.* **95**, 1055–1062 (2006).
43. S. H. Ambrose, L. Norr, "Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate" in *Prehistoric Human Bone: Archaeology at the Molecular Level*, J. B. Lambert, G. Grupe, Eds. (Springer-Verlag, 1993), pp. 1–37.
44. A. Bogaard, T. H. E. Heaton, P. Poulton, I. Merbach, The impact of manuring on nitrogen isotope ratios in cereals: Archaeological implications for reconstruction of diet and crop management practices. *J. Archaeol. Sci.* **34**, 335–343 (2007).
45. A. Bogaard *et al*., Crop manuring and intensive land management by Europe's first farmers. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 12589–12594 (2013).
46. G. J. Van Klinken, M. P. Richards, B. E. M. Hedges, "An overview of causes for stable isotopic variations in past European human populations: Environmental, ecophysiological, and cultural effects" in *Biogeochemical Approaches to Paleodietary Analysis*, S. Ambrose, M. Katzenberg, Eds. (Kluwer Academic/Plenum Publishers, 2000), pp. 39–63.
47. A. K. Styring *et al*., Refining human palaeodietary reconstruction using amino acid $\delta^{15}N$ values of plants, animals and humans. *J. Archaeol. Sci.* **53**, 504–515 (2015).
48. E. J. Guiry, Dogs as analogs in stable isotope-based human paleodietary reconstructions: A review and considerations for future use. *J. Archaeol. Method Theory* **19**, 351–376 (2012).
49. M. Kapcia, A. Mueller-Bieniek, Archaeobotanical analysis of abundant cereal finds from Kraków Nowa Huta Mogiła 62–Getting back to the old story. *Folia Quat.* **86**, 217–231 (2018).
50. A. Mueller-Bieniek *et al*., Spatial and temporal patterns in Neolithic and Bronze Age agriculture in Poland based on the stable carbon and nitrogen isotopic composition of cereal grains. *J. Archaeol. Sci. Rep.* **27**, 101993 (2019).
51. T. H. E. Heaton, Spatial, species, and temporal variations in the $^{13}C/^{12}C$ ratios of C3 plants: Implications for palaeodiet studies. *J. Archaeol. Sci.* **26**, 637–649 (1999).
52. D. G. Drucker, A. Bridault, K. A. Hobson, E. Szuma, H. Bocherens, Can carbon-13 in large herbivores reflect the canopy effect in temperate and boreal ecosystems? Evidence from modern and ancient ungulates. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **266**, 69–82 (2008).
53. M. S. J. Broadmeadow, H. Griffiths, "Carbon isotope discrimination and the coupling of CO_2 fluxes within forest canopies" in *Stable Isotopes and Plant Carbon-Water Relations*, J. R. Ehleringer, A. E. Hall, G. D. Farquhar, Eds. (Elsevier, 1993), pp. 109–129.
54. N. Buchmann, W.-Y. Kao, J. Ehleringer, Influence of stand structure on carbon-13 of vegetation, soils, and canopy air within deciduous and evergreen forests in Utah, United States. *Oecologia* **110**, 109–119 (1997).
55. J. L. Araus *et al*., Identification of ancient irrigation practices based on the carbon isotope discrimination of plant seeds: A case study from the South-East Iberian Peninsula. *J. Archaeol. Sci.* **24**, 729–740 (1997).
56. M. Wallace *et al*., Stable carbon isotope analysis as a direct means of inferring crop water status and water management practices. *World Archaeol.* **45**, 388–409 (2013).
57. V. G. Heptner, A. A. Sludskii, "Carnivora (Hyaenas and Cats)" in *Mammals of the Soviet Union*, V. G. Heptner, N. P. Naumov, Eds. (Smithsonian Institution Libraries and National Science Foundation, 1992), Vol. 2, part 2, pp. 1–784.
58. D. Turner, P. Bateson, Eds., *The Domestic Cat: The Biology of its Behaviour*, (Cambridge Univ. Press, ed. 2, 2000).
59. D. Krauze-Gryz, J. Gryz, J. Goszczyński, Predation by domestic cats in rural areas of central Poland: An assessment based on two methods. *J. Zool. (Lond.)* **288**, 260–266 (2012).
60. Y. Hu *et al*., Earliest evidence for commensal processes of cat domestication. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 116–120 (2014).
61. G. Bar-Oz, L. Weissbrod, E. Tsahar, Cats in recent Chinese study on cat domestication are commensal, not domesticated. *Proc. Natl. Acad. Sci. U.S.A.* **111**, E876 (2014).
62. J. D. Vigne *et al*., Earliest "domestic" cats in China identified as leopard cat (*Prionailurus bengalensis*). *PLoS One* **11**, e0147295 (2016).
63. Y. I. Naito *et al*., Heavy reliance on plants for Romanian cave bears evidenced by amino acid nitrogen isotope analysis. *Sci. Rep.* **10**, 6612 (2020).
64. A. Szczepanek *et al*., Understanding Final Neolithic communities in south-eastern Poland: New insights on diet and mobility from isotopic data. *PLoS One* **13**, e0207748 (2018).
65. G. L. Széles, J. J. Purger, T. Molnár, J. Lanszki, Comparative analysis of the diet of feral and house cats and wildcat in Europe. *Mammal Res.* **63**, 43–53 (2018).
66. K. A. T. Loyd, S. M. Hernandez, J. P. Carroll, K. J. Abernathy, G. J. Marshall, Quantifying free-roaming domestic cat predation using animal-borne video cameras. *Biol. Conserv.* **160**, 183–189 (2013).
67. H. Bocherens *et al*., Paleobiological implications of the isotopic signatures (^{13}C, ^{15}N) of fossil mammal collagen in Scladina cave (Sclayn, Belgium). *Quat. Res.* **48**, 370–380 (1997).
68. S. H. Ambrose, Preparation and characterization of bone and tooth collagen for isotopic analysis. *J. Archaeol. Sci.* **17**, 431–451 (1990).
69. B. Jones, J. Sall, JMP statistical discovery software. *Wiley Interdiscip. Rev. Comput. Stat.* **3**, 188–194 (2011).

70. B. Stock, B. Semmens, *MixSIAR GUI User Manual v3.1*, (Scripps Institution of Oceanography, UC San Diego, 2016).
71. H. Bocherens *et al*., Reconstruction of the Gravettian food-web at Předmostí I using multi-isotopic tracking (^{13}C, ^{15}N, ^{34}S) of bone collagen. *Quat. Int.* **359–360**, 211–228 (2015).
72. C. Wißing *et al*., Stable isotopes reveal patterns of diet and mobility in the last Neandertals and first modern humans in Europe. *Sci. Rep.* **9**, 4433 (2019).
73. C. Baumann *et al*., Dietary niche partitioning among Magdalenian canids in southwestern Germany and Switzerland. *Quat. Sci. Rev.* **227**, 106032 (2020).
74. R. Inger, A. Jackson, A. Parnell, S. Bearhop, *SIAR V4 (Stable Isotope Analysis in R) An Ecologist's Guide*, (University College Dublin, Ireland, 2010).
75. M. T. Krajcarz, M. Krajcarz, H. Bocherens, Collagen-to-collagen prey-predator isotopic enrichment ($\Delta^{13}C$, $\Delta^{15}N$) in terrestrial mammals–A case study of a subfossil red fox den. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **490**, 563–570 (2018).
76. K. Dionne, F. Dufresne, C. Nozais, Variation in $\delta^{13}C$ and $\delta^{15}N$ trophic enrichment factors among *Hyalella azteca* amphipods from different lakes. *Hydrobiologia* **781**, 217–230 (2016).
77. A. Gelman *et al*., *Bayesian Data Analysis*, (Chapman and Hall/CRC, 2014).
78. A. L. Jackson, R. Inger, A. C. Parnell, S. Bearhop, Comparing isotopic niche widths among and within communities: SIBER–Stable isotope bayesian ellipses in R. *J. Anim. Ecol.* **80**, 595–602 (2011).
79. Ø. Hammer, D. A. T. Harper, P. D. Ryan, PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electron.* **4**, 1–9 (2001).
80. J. Dabney *et al*., Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 15758–15763 (2013).
81. M. Kircher, S. Sawyer, M. Meyer, Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Res.* **40**, e3 (2012).
82. M. Baca *et al*., Highly divergent lineage of narrow-headed vole from the Late Pleistocene Europe. *Sci. Rep.* **9**, 17799 (2019).
83. P. J. Reimer *et al*., IntCal13 and Marine13 radiocarbon age calibration curves 0–50,000 Years cal BP. *Radiocarbon* **55**, 1869–1887 (2013).
84. C. Bronk Ramsey, Bayesian analysis of radiocarbon dates. *Radiocarbon* **51**, 337–360 (2009).
85. J. Kruk, Badania poszukiwawcze i weryfikacyjne w górnym i środkowym dorzeczu Szreniawy. *Spraw. Archeol.* **22**, 271–294 (1970).
86. J. Kopacz, A. Pelisiak, Rejon pracowniano-osadniczy and rzeką Krztynią, woj. Częstochowa. *Spraw. Archeol.* **38**, 191–199 (1986).
87. M. T. Krajcarz *et al*., Loess in a cave: Lithostratigraphic and correlative value of loess and loess-like layers in caves from the Kraków-Częstochowa Upland (Poland). *Quat. Int.* **399**, 13–30 (2016).
88. H. Bocherens, D. Drucker, Trophic level isotopic enrichment of carbon and nitrogen in bone collagen: Case studies from recent and ancient terrestrial ecosystems. *Int. J. Osteoarchaeol.* **13**, 46–53 (2003).
89. L. J. Reitsema, T. Kozłowski, Diet and society in Poland before the state: Stable isotope evidence from a Wielbark population (2nd c. AD). *Anthropol. Rev.* **76**, 1–22 (2013).