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Copy Number Variation in the Amylase Gene (*AMY*) in the Brown Bear (*Ursus arctos*), Based on Whole Genome Resequencing Data

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Copy number variation (CNV) in gene loci in animals can be driven by adaption to the environment. The relationship between CNV in genes for amylase (*AMY*), which hydrolyzes starch, and dietary adaptation has been well studied. Copy number (CN) of *AMY* is higher in human populations with high-starch diets, compared with those with low-starch diets. Although CNV in *AMY* has been reported in humans and some domestic animals, there have been few studies of animals in the wild. The brown bear (*Ursus arctos*), widespread in the Northern Hemisphere, shows large dietary variation among individuals and groups. Brown bear population genetic structures are associated with the dispersal history due to climate change over the past few tens of thousands of years, and this together with dietary variation should have led to CNV in *AMY*. In this study, we investigated CNV in *AMY* in brown bears worldwide by using whole genome sequencing data. We detected *AMY* CNV among regional groups. *AMY* CN was similar among brown bears in geographically proximate populations, such as between Hokkaido (Japan) and East Asia, and between the North America mainland and the ABC islands. CNs were smaller in bears from East Asia, including Hokkaido, compared to those from other regions across brown bears' distribution. Our results suggest that CNs of *AMY* reflect the population demographic history of brown bears after the Last Glacial Maximum.

Key words: amylase, brown bear, copy number variation, population demography, whole genome sequencing

INTRODUCTION

Genetic variation, including single nucleotide polymorphisms (SNPs) and structural variation in nucleotide sequences, is related to adaptation to the environment. One aspect of genetic diversity is copy number variation (CNV), which is genomic structural variation involving duplications of a gene locus (Mills et al., 2011). CNV in animals can occur due to adaptation to the environment. For example, duplications of the fatty acid desaturase gene *Fads2* allow originally marine fishes to colonize freshwater environments (Ishikawa et al., 2019), suggesting maintenance of differences in copy

number (CN) between fishes living in freshwater and saltwater. The relationship between CNV and adaptation has also been suggested in mammals. Lower CNs of the CC chemokine gene *CCL3L1*, which encodes a ligand for the major coreceptor for HIV and a major HIV-suppressive chemokine, were associated with high HIV/acquired immunodeficiency syndrome (AIDS) susceptibility (Gonzalez et al., 2005). Another example is bitter taste gene *TAS2Rs*: Marine mammals (Cetacea and Pinnipedia), which swallow prey whole, showed lower CNs of *TAS2Rs*, while insectivorous animals such as the shrew and insectivorous bats had higher CNs (Liu et al., 2016). Changes in copy number can affect gene expression and enzyme activity, both of which influence enzymatic work or the enzymatic synthesis. For example, CN of the amylase gene (*AMY*) in domestic dogs is corre-

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lated with gene expression and amylase activity (Axelsson et al., 2013). CNV can thus affect the phenotype and play an important role in adaptation to the environment.

AMY has been well studied for the relationship between CN and dietary phenotypes. Alpha-amylase hydrolyzes the α -(1→4) linkage in starch chains (Robyt, 2008). In humans, the CN of *AMY* tends to be higher in populations with high-starch diets compared with those with low-starch diets, suggesting a connection between CN and adaptation (Perry et al., 2007; Bolognini et al., 2024). Similarly, the CN of *AMY* is greater in domestic dogs than in wolves, which might be related to domestication and adaptation to starch-rich diets in dogs (Axelsson et al., 2013).

Although CNV in *AMY* has been extensively studied in humans and some domestic animals such as dogs, pigs, mice, and rats (Pajic et al., 2019), there have been few studies of animals in the wild. Based on a read-depth approach using whole genome resequencing data, Rinker et al. (2019) reported that the CN of *AMY1B* was lower in polar bears (*Ursus maritimus*) than in brown bears (*Ursus arctos*) from North America and Europe. Polar bears are almost exclusively carnivorous and depend on seals (Derocher, 2012; Iversen et al., 2013), whereas brown bears are omnivorous and eat plants such as nuts and berries, which contain a significant amount of starch (Paralikiadis et al., 2010; Fortin et al., 2013). This could have driven the difference in CN of *AMY1B* between polar bears and brown bears after their divergence. Although CNV of *AMY* has not yet been studied among brown bear populations, high variation is expected because the diet varies among regional groups (Clevenger et al., 1992; Hilderbrand et al., 1999; Sato et al., 2005). Brown bears from North America tend to have a diet with high portion of salmon, with meat consumption around 80% (Hilderbrand et al., 1999), while bears from Europe (Spain) do not eat meat so much (Clevenger et al., 1992). If CNs of *AMY* reflect these diets' trends, bears from North America would possess small CNs and ones from Europe would possess large CNs. It was reported that the consumption of salmon in Hokkaido brown bears declined in the last 200 years (Matsubayashi et al., 2015). Therefore, Hokkaido brown bears are expected to have lower CNs of *AMY*, if these CNs reflect the adaptation to starch-rich diet. Diets of brown bears in Tibet, where the climate is characterized by dry, cold winters, were reported to consist of meat of mammals with a large frequency occurrence during summer (Aichun et al., 2006). Additionally, the diets of brown bears in North America vary within a single population (Edwards et al., 2011). As a result, CNV of *AMY* is expected to be observed within populations.

Worldwide, brown bears have complex population genetic structures and demographic histories, and show genetic diversity. In a study of autosomal genomes, de Jong et al. (2023) found that Eurasian brown bears are divided mainly into western and eastern clades whose boundary lies west of the river Yenisei in western Siberia. Bear groups are named according to geographic region and include the Middle Eastern, Himalayan, Western Eurasian (western clade), Eastern Eurasian and Hokkaido (eastern clade), Kodiak, and North American groups (de Jong et al., 2023). Previous studies likewise revealed discontinuous distributions for mitochondrial DNA (mtDNA) lineages. For example,

the clade 4 lineage was detected only in Southern Hokkaido and North America (Hirata et al., 2013; de Jong et al., 2023). These mtDNA lineages migrated at different times via land bridges (e.g., between the continent and Sakhalin, and between Sakhalin and Hokkaido) formed by past sea-level changes. In particular, mtDNA clade 3a1 dispersed widely to Eurasia after the last glacial maximum (LGM) (Korsten et al., 2009). Polar bears fall within the same mtDNA lineage as brown bears in the Admiralty, Baranof, and Chichagof (ABC) islands in Alaska (Talbot and Shields, 1996; Hirata et al., 2013). In addition, the genome of brown bears in the ABC islands have many traces of introgression from the polar bear genome (Cahill et al., 2013, 2015). The hybridizations of brown bears with polar bears possibly affected the difference of mtDNA between the brown bear and polar bear (Hailer et al., 2012; Miller et al., 2012). Thus, the CNs of polar bear *AMY* are expected to be more similar to those of brown bears in the ABC islands than to those that of brown bears on the North America mainland.

In this study, we analyzed CNV in *AMY* for brown bears worldwide using whole genome sequencing data. These results can contribute to understanding patterns of diversity and processes of intraspecific evolution in brown bears.

MATERIALS AND METHODS

Samples and mapping

Whole genome resequencing data from 49 brown bears, 10 polar bears, one American black bear (*Ursus americanus*), and two Asiatic black bears (*Ursus thibetanus*) were obtained from the NCBI databank and used in this study (see Supplementary Table S1). From nine samples (AA1, ETF1, U05M, U12M, U13M, U34, U40, U47M, and U76), the total DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) and stored at 4°C or 20°C until used. Then, 150-bp pair-end whole-genome resequencing of these samples was performed on an Illumina Novaseq 6000 sequencer by MacroGen Japan (Tokyo, Japan). The libraries were prepared using the TruSeq DNA Nano Sample Prep Kit (Illumina, San Diego, USA). The average total amount of sequence data per sample ordered was 90 Gb. Figure 1 shows sampling locations and groups.

After the quality of sequences was checked with FastQC ver. 0.11.8 (Babraham Bioinformatics, 2011) and poor-quality sequences trimmed with Trimmomatic ver. 0.39 (Bolger et al., 2014) with the option "LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36," reads were mapped to the polar bear reference genome (ASM1731132v1, accession number: GCF_017311325.1) by using the BWA-MEM algorithm implemented in BWA ver. 0.7.17 (Li and Durbin, 2009) with the '-M' command option. The percentage of reads for which both the first and second reads in a pair aligned to the reference sequence was calculated using picard.jar (Broad Institute, 2019).

Identifying amylase regions in the polar bear reference genome

The reference genome was searched for *AMY* positions. We extracted the coding sequences (CDSs) from the coding sequence file, which were annotated as "AMY1B" or "AMY2B" in the annotation GFF file downloaded from GigaDB (<http://gigadb.org/dataset/100008>). Amylase regions in the reference genome were found by using CDS data as queries in blastn searches using BLAST ver. 2.15.0. Two regions matching the CDS data that corresponded to the amylase-like genes in the polar bear reference genome were identified and are here referred to as AMY-1 and AMY-2.

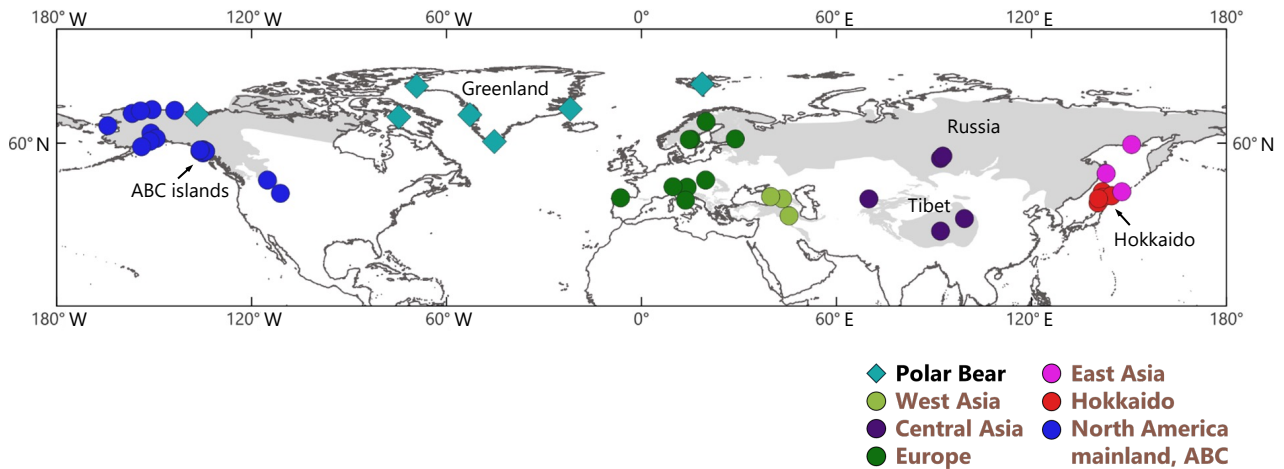


Fig. 1. Geographic distribution of bear samples used in this study. Gray shading shows the range of brown bears, based on McLellan et al. (2017). Symbols indicate sample localities; circles, brown bears; diamonds, polar bears. In the key at lower right, circle colors indicate brown bear groups (brown font), corresponding to those listed in Supplementary Table S1.

Reconstruction of a phylogenetic tree for mammalian amylase genes

Rinker et al. (2019) showed a CN difference between *AMY1* and *AMY2* and then discussed how the difference correlated with function. However, correlating a difference in copy number with gene function might not be valid, because Pajic et al. (2019) showed that *AMY* in mammals diverged independently in each species. To clarify the phylogeny of amylase-like genes, a phylogenetic tree was constructed for *AMY* from representative mammals, including polar bears. We downloaded 70 amino acid sequences for 30 *AMY* genes (see Supplementary Table S2) from the NCBI database and then conducted a maximum likelihood analysis of these sequences. After alignment using the MAFFT algorithm (Katoh et al., 2002), sites including one or more gaps were excluded by using Unipro UGENE (Okonechnikov et al., 2012). A maximum likelihood tree based on 272 amino acid positions was constructed with IQ-tree2 (Nguyen et al., 2015) under the options -m MFP, -bb 1000, -alrt 1000, -nt AUTO. Nodal support was assessed by analysis of 1000 bootstrap pseudoreplicates by using an amino acid exchange-rate matrices model, the Revised JTT Matrix model (Kosiol and Goldman, 2005), based on the Bayesian information criterion values.

Estimation of CNs of *AMY* in different populations

CNs were estimated for *AMY* by using the read depth-based method (Yoon et al., 2009). The target region was defined for each of *AMY1* and *AMY2* in the reference genome. These regions included both CDSs and introns found by homology searches. The *AMY2* region contained two isoforms, XM_040626972.1 and XM_040626973.1, but our results include only the region corresponding to XM_040626973.1. We also analyzed the alternative transcript region XM_040626972.1, but the results obtained were essentially the same as those with XM_040626973.1 (data not shown).

For each sample, a copy-invariant region was defined in which the mappability equaled 1 and no gene was included. The mappability was calculated with GenMap ver. 1.3.0 (Pockrandt et al., 2020) under the condition that the 30-mer starting at each nucleotide site occurred only once in the sequence, with two or fewer errors, under the parameters -K 30 -E 2 -t -w -bg. Reads of these regions were counted for each sample by using SAMtools ver. 1.7, with samtools view (Li et al., 2009). We tested two ways of counting reads in target regions: 1) reads were counted throughout the target region (*AMY1* and *AMY2* in Fig. 2), and 2) reads were counted in parts of the tar-

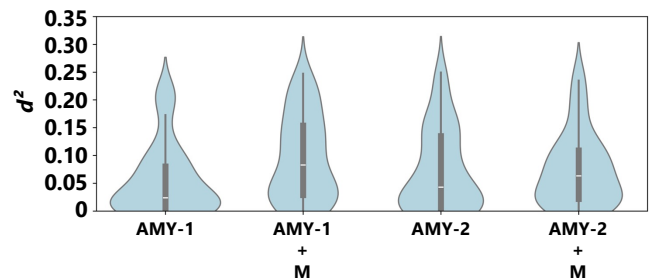


Fig. 2. Violin plots of d^2 calculated for *AMY1* and *AMY2* regions to count reads. Each light blue shape shows a kernel density estimate. “+ M” means that reads were counted in regions where the mappability equaled 1.

get region in which the mappability equaled 1 (*AMY1* + M and *AMY2* + M in Fig. 2). The mapping density (MD) was calculated with the following equations (Carpenter et al., 2015).

$$MD_{\text{target}} = \frac{\text{reads mapped in target region}}{\text{target region size}}$$

$$MD_{\text{control}} = \frac{\text{reads mapped in copy-invariant region}}{\text{copy-invariant region size}}$$

$$MD_{\text{normalized}} = \frac{MD_{\text{target}}}{MD_{\text{control}}}$$

CN was calculated by rounding $2MD_{\text{normalized}}$, which is expected to be a natural number. However, decimal numbers could arise due to bias in the number of reads per region. To evaluate decimals, we defined d^2 as the square of decimals omitted by rounding.

$$d^2 = (2MD_{\text{normalized}} - \text{CN})^2$$

CN was correctly estimated as d^2 was smaller. CN was compared among groups of bears by region by means of Tukey's Honestly Significant Difference (HSD) test using the package *statsmodels* ver. 0.14.0 (Seabold and Perktold, 2010) in Python.

Estimation of CN based on the read counts per 100 bp

To reveal partial increases in *AMY*, CN per 100 bp was estimated. Two samples from each group were chosen for this analysis (see Supplementary Table S1). We chose the samples that showed near average values of CNs in *AMY*-2 in the results of the above estimation. We calculated CN per 100 bp as follows:

$$\text{CN per 100 bp} = \frac{\text{reads mapped in 100 bp region} / 100}{\text{reads mapped in whole reference genome} / \text{whole reference genome size}}$$

RESULTS

Identifying amylase regions in the polar bear reference genome and reconstruction of a phylogenetic tree for mammalian amylase genes

BLAST searches for *AMY* regions using the coding sequence data of the reference genome detected one *AMY2B* and two *AMY1B* regions in the polar bear genome (*AMY2B*, LOC103669312; *AMY1B*, LOC103669314). The direction of *AMY1B* was the same as in the human (hg38) reference assembly.

In our phylogenetic tree of *AMY* sequences from mammals (see Supplementary Figure S1), *AMY1* and *AMY2* are not separated into different lineages; instead, *AMY* amino acid sequences from mammals are divided by species. Our study determined polar bear LOC103669312 to be *AMY*-1 and LOC103669314S to be *AMY*-2 and did not discuss the result correlating with the difference in *AMY* function.

CN estimation by the read depth-based method

We calculated d^2 for each sample (Fig. 2). CN is better estimated by the method that yields a smaller d^2 value. Judging by the shapes and median values of the violin plots

for both *AMY*-1 and *AMY*-2, counting reads without considering the mappability was more reliable than counting reads in the regions in which the mappability was 1. Here, we present the results obtained without considering the mappability; those in which mappability was taken into account are shown in Supplementary Figure S2.

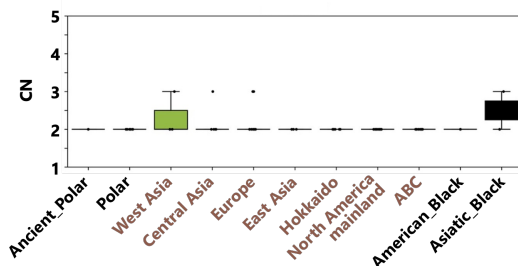
CNV estimation

No significant differences in CN were found among the populations for *AMY*-1. Samples grouped by geographical region showed a CN of two or three (Fig. 3A). Tukey's HSD test did not detect a significant difference in CN for *AMY*-1 among bear species or brown bear populations (see Supplementary Table S3).

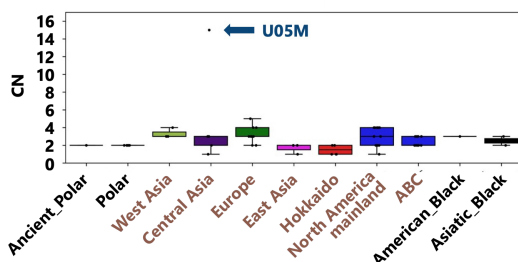
CN estimates for *AMY*-2 without considering the mappability were higher for the populations of brown bears from West Asia (mean = 3.3), Central Asia (mean = 4.8), Europe (mean = 3.2), the North America mainland (mean = 2.9), and the ABC islands (mean = 2.4) than for polar bears (mean = 2) (Fig. 3B), though the differences were not statistically significant (see Supplementary Table S4). On the other hand, brown bears from East Asia (mean = 1.7) and Hokkaido (mean = 1.5) showed low *AMY*-2 CNs.

CNs for *AMY*-2 were similar between the North America mainland and ABC populations, and between the Hokkaido and East Asia (including samples from Sakhalin and Etorofu [Iturup]) populations (Fig. 3C). This makes sense geographically, as the ABC islands are located in Alaska (North America), and Hokkaido, Sakhalin, and Etorofu (Iturup) are located in East Asia. The Mantel test showed a statistically significant positive correlation between the difference of *AMY*-2 CNs and geographical difference between individuals (Mantel statistic $r = 0.168$, $P = 0.0026$). Among brown

A. *AMY*-1



B. *AMY*-2



C. *AMY*-2 (Without U05M)

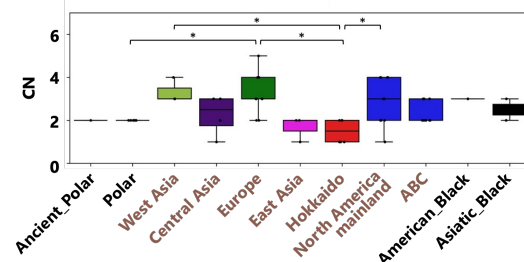


Fig. 3. (A–C) Box plots of CN for *AMY*-1 and *AMY*-2 by species and region; CN was estimated without considering the mappability. The whiskers extend to 1.5× the interquartile ranges (IQRs) of the lower and upper quartiles. Brown bear group names are in brown font.

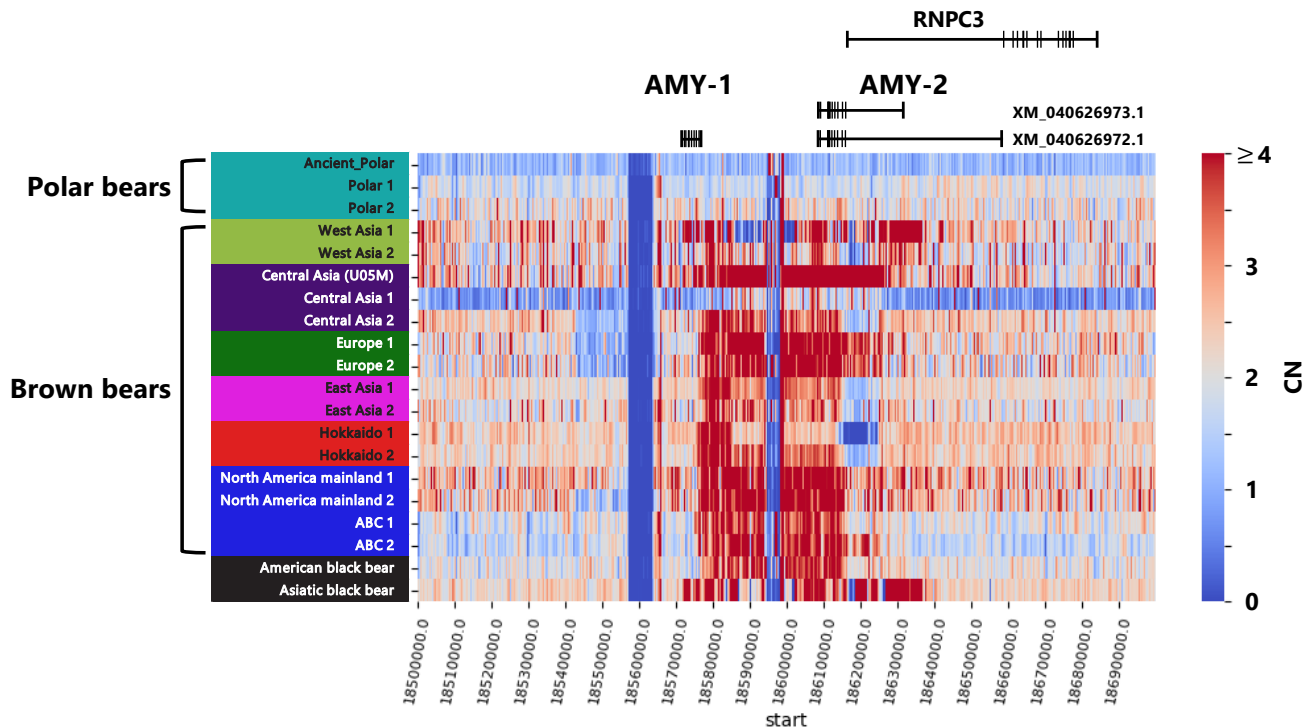


Fig. 4. Heat map for CNs calculated based on read counts per 100 bp around the *AMY* region in the reference genome. The samples used for the heat map are listed in Supplementary Table S1.

bears examined, U05M from Central Asia (see Supplementary Table S1) showed a much higher CN (15) for *AMY*-2 than did other bears. We considered the possibility of sequencing errors in U05M, because this was an old sample (Hirata et al., 2014). An analysis with sample U05M removed (Fig. 3C) showed brown bears on the North America mainland and in Europe to have significantly higher CNs for *AMY*-2 than bears on Hokkaido Island, Japan ($P = 0.0199$ and 0.0037 , respectively, Tukey's HSD test, see Supplementary Table S5). Compared to CNs of brown bears in East Asia including Hokkaido, CNs of bears on the North America mainland and Europe were also significantly higher than those in East Asia including Hokkaido ($P = 0.0056$ and 0.0008 , respectively, Tukey's HSD test). In addition, CN was significantly higher for brown bears in Europe than for polar bears ($P = 0.0467$, see Supplementary Table S5).

The degree of CNV was large within bear populations of Central Asia, Europe, and North America mainland, respectively.

CNs based on read counts per 100 bp

Partial DNA segment was duplicated even within the genic region of *AMY* (Rinker et al., 2019). If specific exons duplicate and come to have new functions, the exons could work differently or be separated to use through alternative splicing. To reveal whether partial DNA segments increased, CNs were estimated based on read counts per 100 bp in the region around *AMY* (Fig. 4). The duplication of partial DNA segments was observed in both exons and introns, and there seemed to be no relationship between CNs in exon and intron regions. The genomic regions showing CNs of *AMY*-2 were variable within populations, especially from

Central Asia and the ABC islands.

DISCUSSION

We examined CNV for *AMY* in brown bears worldwide. Because brown bears utilize a variety of diets and show genetic diversity, it is reasonable to expect CNV in *AMY*. CN increased partially across the entire locus, including in exons and introns, rather than in a specific exon (Fig. 4). This suggests that CN did not increase solely in an exon encoding a specific enzymatic functional region. Sometimes specific exons duplicate and come to have new functions. In that case, exons would work differently or be separated to use through alternative splicing.

To estimate exact CNs for *AMY*, we counted reads of the target region in the reference genome, with and without considering the mappability. CN was thought to have been estimated correctly, because d^2 (defined as the square of decimals omitted by rounding) was smaller when the mappability was not considered (Fig. 2) than when it was. Mappability refers to the uniqueness of the genome. Regions showing a mappability of 1 are not repetitive, so reads can be mapped correctly in these regions. It is unknown why d^2 was smaller when the mappability was not taken into account. However, the number of sites to analyze decreased in regions where the mappability was 1, so CNs were not estimated correctly when the mappability was considered.

Our study demonstrated CNV of *AMY*-2 within brown bears for the first time. We revealed that *AMY*-2 of brown bears from West Asia, Central Asia, Europe, the North America mainland, and the ABC islands had higher CNs than polar bears. As shown in Fig. 3B, CN was unusually high (15) for one Tibetan bear (U05M), due either to idiosyn-

cratic duplications in this individual or to technical or artifactual issues, such as when creating a library. U05M was an old sample (Hirata et al., 2014), and the DNA in such samples can undergo fragmentation, leading to biases in sequencing. Additionally, 15 is large as an *AMY* CN value compared to other mammalian *AMY* CNs (Pajic et al., 2019). Considering that the high CN for U05M might be an error, we also analyzed the data with U05M removed (Fig. 3C). This analysis showed brown bears on the North America mainland, in Europe, and in West Asia to have higher CN than those in East Asia and Hokkaido. However, the experimental condition of U05M was the same as that of the other eight samples used in Endo et al. (unpublished data) and there was not much difference in indexes such as mapping rates between U05M and other samples (see Supplementary Table S1). Therefore, it remains possible that CNs of U05M reflect the increase of CNs. Thus, additional analyses using additional current samples from the same region are needed.

The CN of *AMY*-2 was similar between brown bears from geographically close locations, such as between Hokkaido and East Asia, and between the North America mainland and ABC islands (Fig. 3C). Although polar bear DNA introgression into the ABC islands brown bear genome was reported (Cahill et al., 2013, 2015), *AMY*-2 CN of ABC brown bears was not similar to that of polar bears. Rather, our results likely reflect the effects of isolation by distance. As previous studies (Endo et al., 2021; de Jong et al., 2023) have shown that isolation by distance can affect brown bears, admixture likely occurred between geographically proximate populations isolated by distance from other populations. Additionally, differences in vegetation and food resources could potentially explain our results. Because brown bear diets can be affected by climatic factors (Bojarska and Selva, 2012), the diets could be similar between geographically close locations which have similar environmental conditions. In fact, the current vegetation in Hokkaido and Sakhalin is similar: evergreen conifer forests of *Picea jezoensis* and *Abies sachalinensis* (Igarashi, 2016), while Alaska is occupied by boreal forests (e.g., *Picea mariana*) and tundra (Anderson and Brubaker, 1994). Food resources are also different between geographically distant places. Although herbaceous plants were the dominant food in spring both in Hokkaido and in Europe, berries (e.g. *Actinidia arguta*) were consumed more than acorns and nuts by Hokkaido bears in autumn (Sato et al., 2005), while hard mast such as

Oak acorns was greatly eaten in autumn by European bears (Clevenger et al., 1992).

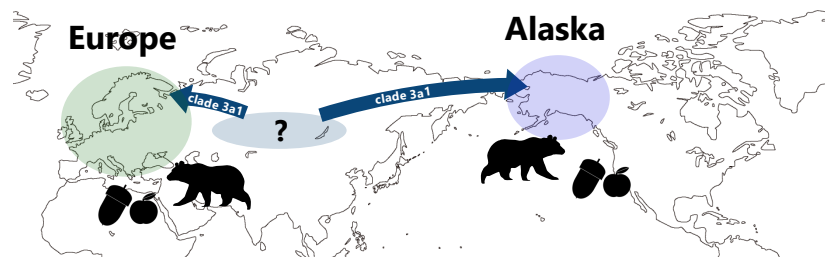
The degree of CNV within a single population has been observed largely in Central Asia, Europe, and North America's mainland. Previous studies reported that diets of brown bears in North America varied within a single population (Edwards et al., 2011); therefore, our results were reasonable. However, Tibetan bears had various CNs though they were reported to eat almost entirely meat in summer (Aichun et al., 2006). Therefore, CNV within a single population might not necessarily reflect various diets within a population.

A relationship between elevated CNs of *AMY* and dietary adaptation to a starch-rich diet has been reported in humans (Perry et al., 2007) and dogs (Axelsson et al., 2013). Compared to these mammals, the increases in CN in brown

A. LGM



B. after LGM



C. Present

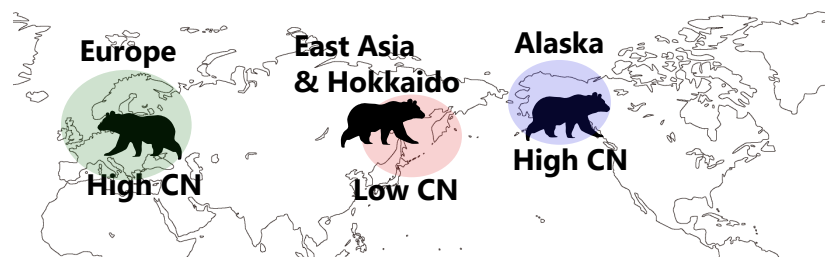


Fig. 5. A schematic representation of diet, migration, and CN of *AMY* for brown bears (**A**) at the LGM, (**B**) after the LGM, and (**C**) now. (**A**) The diet of brown bears tended to be carnivorous at the LGM. (**B**) Brown bears migrated to Alaska and Europe after the LGM and their diet shifted to become predominantly herbivorous. (**C**) Brown bears in Europe and Alaska now have high CNs for *AMY* and those in East Asia, including Hokkaido, have low CNs for *AMY*.

bears were not so high. In the Hokkaido brown bear population, consumption of salmon has decreased over the past 200 years (Matsubayashi et al., 2015). If CNs reflect this latest dietary shift, CNs of AMY-2 could have increased in Hokkaido brown bears; however, the CN values (1 in three samples and 2 in three samples) were still low (Fig. 3C). Two hundred years may be too short a time to accrue increased CNs in the Hokkaido bear population. In addition, although we expected that CNs of bears in North America would be low and those in Europe would be high, the CNs of AMY-2 in both regions were high in our results. Such CNV could have resulted from the fact that the differences of diets among individuals are dependent on the seasons and the availability of food resources.

On the other hand, CNs likely reflect the demography of brown bears (Fig. 5). Previous analyses of mtDNA and the nuclear genome have indicated that brown bears spread across the Northern Hemisphere after the LGM (Korsten et al., 2009; de Jong et al., 2023). The distribution of mtDNA clade 3a1 suggests that many individuals migrated to Alaska and Europe (Korsten et al., 2009; de Jong et al., 2023). After the LGM, the diet of brown bears shifted from carnivorous to herbivorous tendencies (Ersmark et al., 2019; Rey-Iglesia et al., 2019). Although our results do not suggest a rapid change in the diet of the Hokkaido bear population in the last 200 years, CN may be affected by diet changes over a larger time scale, for example, across thousands of years after the LGM. Our study newly found that CNs of AMY-2 from North America and Europe were high and those from East Asia were low, which implies that the CN of AMY-2 increased in some brown bears because their diet became increasingly herbivorous over tens of thousands of years, and that some populations with larger CNs for AMY-2 then immigrated to Alaska and Europe. Hence, high CNs of AMY-2 could have spread among populations. Further studies are needed to reveal the relationship between CNs of AMY-2 and the expression and activity of amylase for understanding the adaptation and evolution of brown bears.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

NK conducted analyses of whole genome data and wrote the first draft. YE designed the study, mapped the genome data, and constructed the phylogenetic tree. NO provided advice on analyses. TM and AVA provided samples. RM oversaw the collaboration and corrected the draft manuscript. All authors discussed the draft and have read the final version of the manuscript.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online. (URL: <https://doi.org/10.2108/zs240087>)

Supplementary Table S1. Brown bear (*Ursus arctos*), polar

bear (*Ursus maritimus*), American black bear (*Ursus americanus*), and Asiatic black bear (*Ursus thibetanus*) samples used in this study. Estimated CN for AMY-1 and AMY-2 indicates the results of the values of CN estimation in this study.

Supplementary Table S2. List of source data for AMY amino acid sequences taken from the NCBI database and used for the phylogenetic analysis.

Supplementary Table S3. The results of Tukey's HSD test of AMY-1 CN.

Supplementary Table S4. The results of Tukey's HSD test of AMY-2 CN.

Supplementary Table S5. The results of Tukey's HSD test of AMY-2 CN of samples removed U05M.

Supplementary Figure S1. Maximum-likelihood phylogenetic tree for AMY genes from selected mammals, based on amino acid sequences (272 positions). Samples used to construct the tree are listed in Supplementary Table S2. The monophyletic group indicated in red comprises all AMY genes included for polar bears.

Supplementary Figure S2. Box plots of CN for AMY-1 and AMY-2 by species and region; CN was estimated with the mappability taken into account. The whiskers extend to 1.5× the interquartile ranges (IQRs) of the lower and upper quartiles. Brown bear group names are in brown font.

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