Hindawi
International Journal of Zoology
Volume 2024, Article ID 8445492, 10 pages
https://doi.org/10.1155/2024/8445492

Research Article

Severe Enamel Defects in Wild Japanese Macaques

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Received 25 September 2023; Revised 27 November 2023; Accepted 26 December 2023; Published 10 January 2024

Academic Editor: Edson Gandiwa

Plane-form enamel hypoplasia (PFEH) is a severe dental defect in which large areas of the crown are devoid of enamel. This condition is rare in humans and even rarer in wild primates. The etiology of PFEH has been linked to exposure to severe disease, malnutrition, and environmental toxins and associated with systemic conditions. In this study, we examined the prevalence of enamel hypoplasia in several populations of wild Japanese macaques (*Macaca fuscata*) with the aim of providing context for severe defects observed in macaques from Yakushima Island. We found that 10 of 21 individuals (48%) from Yakushima Island displayed uniform and significant PFEH; all 10 specimens were from two adjacent locations in the south of the island. In contrast, macaques from other islands and from mainland Japan have a low prevalence of the more common types of enamel hypoplasia and none exhibit PFEH. In Yakushima macaques, every tooth type was affected to varying degrees except for first molars and primary teeth, and the mineral content of the remaining enamel in teeth with PFEH was normal (i.e., no hypo- or hypermineralization). The aetiology of PFEH might be linked to extreme weather events or high rates of environmental fluoride-causing enamel breakdown. However, given that the affected individuals underwent dental development during a period of substantial human-related habitat change, an anthropogenic-related etiology seems most likely. Further research on living primate populations is needed to better understand the causes of PFEH in wild primates.

1. Introduction

Enamel hypoplasia is a common type of defect in which there is reduction in enamel volume caused by cessation or diminution of ameloblast function [1–3]. Defects are often characterized into one of four categories: linear-form (LEH), pit-form (PEH), plane-form (PFEH), and localized enamel hypoplasia [1, 4–7]. The classification of defects into these categories can sometimes be difficult, e.g., [7–10]. Enamel hypoplasia has been commonly recorded and described in archaeological and paleontological studies, with the "health" or "stress" status of populations or taxa often assessed using hypoplasia as a proxy, e.g., [2, 11–13]. The use of enamel hypoplasia as a tool to understand physiological disturbances to recent or contemporary populations has received much less attention, e.g., [14], and its potential application to help assess habitat and environmental impacts remains relatively unexplored. Here, we investigate a severe type of enamel hypoplasia and explore the information it may provide about a population of macaques born during a period of substantial human-induced habitat change on the island of Yakushima, Japan.

PFEH is one of the most severe and rarest types of enamel hypoplasia, resulting from a complete cessation of enamel matrix formation that results in large areas of the tooth crown having little or no enamel deposition [1, 7, 8, 15–17]. In humans, severe tooth defects in which large areas of enamel do not form correctly often relate to diseases and environmental factors such as localized trauma, congenital syphilis, medical treatments associated with the use of mercury, and specific genetic conditions such as amelogenesis imperfecta [8, 9, 17–19]. Such severe dental defects, like PFEH, are rarely observed in wild primates [10].

Less severe enamel defects, such as localized enamel hypoplasia, linear enamel hypoplasia (LEH), and pit-form hypoplasia (PEH) are, in contrast, relatively common in wild primates, e.g., [3, 7, 20–22]. The prevalence and severity of these defects vary between primates, not just based on illness, malnutrition, and disease susceptibility but also relating to tooth developmental timing and morphological/structural differences among teeth [3, 7]. However, unlike these other types of enamel hypoplasia, PFEH is considered an extreme defect thought to be associated with life-threatening periods of malnutrition, disease, or chemical exposure [8, 9, 17–19]. These defects, when observed in nonhuman primates, may offer unique insight into how different primates cope with extreme physiological stress, which may include anthropogenic-related changes to their habitat and environment.

In this study, skeletal remains of Japanese macaques (*Macaca fuscata*) from a range of locations were examined for evidence of macroscopically visible enamel defects. All types of enamel defects were recorded, including LEH, PEH, PFEH, and localized defects. A special focus was placed on individuals from the South of Yakushima Island that underwent dental development in the 1980s, since severe defects in multiple contemporary individuals were observed during data collection. The frequency and severity of these defects were compared to those in macaques from other geographic regions, and a differential diagnosis of PFEH was undertaken, including assessment of affected teeth and potential hypo/hypermineralization of the remaining enamel. We hypothesize a severe environmental event might be linked to the aetiology of PFEH in these individuals.

2. Materials and Methods

Specimens studied originate from three Japanese islands and the mainland (Yakushima: 21 individuals; Honshu: 10 individuals; Koshima: 19 individuals). All specimens are curated at the Primate Research Institute (PRI) (now the Center for the Evolutionary Origins of Human Behavior), Kyoto University, Japan. All 48 individuals studied lived in the wild, with those on Koshima (Kojima) Island provisioned regularly as part of a primatological study [23, 24]. Here, we focused on individuals from Yakushima Island. Yakushima macaques are often considered a subspecies of Japanese macaques (*Macaca fuscata yakui*), although this is debated, especially in light of genetic evidence [25, 26]. The macaques studied originate from different areas of the island (Figure 1), with males and females studied (5 males; 8 females; 8 unknown). Most individuals lived and died in the 1980s. Table 1 lists samples in more detail, including the location in which they were collected.

Enamel hypoplasia data were collected following Towle and Irish [27] and are briefly summarized here. Teeth were held under a lamp and rotated, allowing light to hit the surface at different angles. The smallest discernible macroscopic defect was recorded, with a hand lens used to rule out postmortem damage. Postmortem damage was distinguished from enamel hypoplasia by distinct characteristics, including sharp edges and contrasting coloration between the fractured enamel and the rest of the crown. These features seldom resemble common forms of enamel hypoplasia, such as pitting, linear, or plane-form defects. Under a hand lens, postmortem damage also lacks evidence of wear during the individual's life. Methods for recording LEH follow Goodman and Rose [28] and Miszkiewicz [29]. Localized hypoplasia was recorded following Skinner et al. [6]. PEH was recorded if there were multiple circular/oval enamel defects on a tooth crown [27]. If pitting was present within a LEH band, then it was recorded as LEH not PEH, but the pitting was noted. Plane-form enamel hypoplasia was recorded following Towle et al. [17]. Data are presented by tooth count rather than individual, with the number of hypoplastic teeth displayed as a percentage of the total number of observable permanent teeth.

To assess for evidence of dental tissue mineralization changes associated with PFEH, mineral concentration (MC) was calculated using microcomputed tomography (micro-CT) on two teeth of an individual with PFEH (a lower first molar without defects and a second molar with PFEH) and a tooth from an individual with no PFEH. Scans were undertaken at the PRI using a SkyScan1275 micro-CT scanner. X-rays were generated at 100 kV, 100 μA, and 10 W, with a 1 mm copper filter placed in the beam path. Resolution was set at 15 μm voxels, and rotation was set to 0.2 degrees. Images were reconstructed using the Skyscan NRecon software (NRecon, version 1.4.4, Skyscan) with standardized settings (smoothing 3; ring artifact correction 10; beam hardening 30%). Resin-hydroxyapatite phantoms were used to calibrate grayscales and mineral densities in each specimen [30]. The calibration methods followed Schwass et al. [30]; Loch et al. [31]; Towle et al. [32]; and Towle et al. [33]. Average values were calculated for each individual tooth by recording MC at three locations (oval ROI: 0.15 mm diameter) across the enamel thickness (outer enamel, middle enamel, and inner enamel) at four crown locations (buccal, lingual, distal, mesial). For this, a single slice was selected just above the point at which the pulp becomes visible. The average value for these 12 ROI was then calculated to give the specimen average. This standardized location was chosen because a comparison of full crown MC is not feasible in teeth with PFEH.

The data analyzed for this study are openly available in Dryad (doi: 10.5061/dryad.6t1g1jx46), which also includes the location, year of inclusion in the PRI collection, and sex of each individual studied when it was available. Sex assignment was taken from the PRI database, and in the present study, we did not attempt to assign sex to the remaining specimens. Further details on the samples, as well as other information on tooth wear and pathologies for these populations can be found in Towle et al. [24] and Towle and Loch [34]. The analysis of the morphological defects employed here was descriptive.

3. Results

Only individuals from Yakushima Island have PFEH, with 68 teeth demonstrating this defect. A total of 10 out of 21 individuals (48%) show PFEH. Most individuals with PFEH

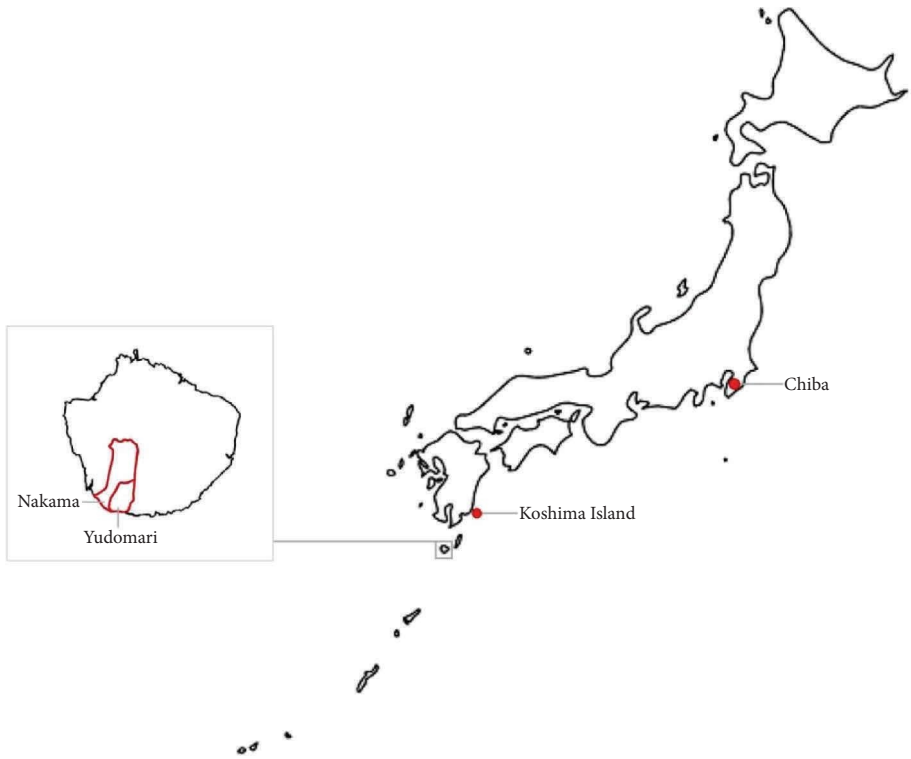

Figure 1: Map of Japan, with the three islands/areas from which the studied macaques lived indicated (Chiba, Koshima Island, and Yakushima Island). The two adjacent areas on Yakushima Island in which individuals with plane-form enamel hypoplasia are found are highlighted, Yudomari and Nakama.

Table 1: Information on the samples studied from Yakushima Island, including sample number, sex, year of death/collection/reception, and locality (translated).

Sample	Sex	Reception date	Locality on Yakushima Island
9952	Male	2010	Not stated
9013	Female	2009	Han-yama
10377	Female	2014	Not stated
2593	Male	1987	Nakama
2595	Female	1987	Kusugawa
2603	Not known	1987	Nakama
3130	Female	1989	Yudomari
3133	Not known	1989	Yudomari
2600	Female	1987	Nakama
2598	Female	1987	Nakama
3139	Not known	Not known	Yudomari
3158	Not known	1989	Isso
3430	Female	1989	Han-yama
1730	Male	1983	Not stated
2597	Female	1987	Nakama
2604	Not known	1987	Nakama
3124	Male	1989	Yudomari
3136	Not known	1989	Yudomari
3138	Male	1989	Yudomari
3435	Not known	1989	Yaku-cho
3434	Not known	1989	Yaku-cho

Reception date relates to the date that the PRI study material committee received the specimen or registered it to the database.

lived in the neighboring areas of Yudomari (PRI specimen numbers: 3130, 3133, 3139, 3124, 3136) and Nakama (PRI specimen numbers: 2600, 2598, 2604) in the south of the island (Figure 2). Two other individuals displaying PFEH (PRI 3435 and 3434) likely came from this same area, although a precise location was not recorded. Extreme wear

obscured the crown surfaces enough to make it difficult to infer PFEH status for another individual from this locality (PRI 3138), but given the presence of atypical wear that matches expectations of PFEH (first molar less worn than second and third molars) and a large number of dental abscesses, we presume this individual had PFEH earlier in life. In particular, such extreme wear on the second and third molars, but normal wear on the first molars, cannot be explained by other processes. This may also be the case for the other individuals in Yudomari and Nakama, with advanced wear potentially erasing evidence of previous defects. Other individuals from these two areas also display other types of enamel hypoplasia (PRI specimens: 2593, 2603, 2597). In individuals from other locations, both on Yakushima Island, Honshu, and Koshima, no cases of PFEH were observed (i.e., 0/638 teeth for Chiba and Koshima samples combined). Other types of enamel hypoplasia were also more frequent in Yakushima Island individuals compared to Koshima Island and Chiba, including localized defects and LEH (Table 2). No individuals analyzed from any location displayed pitting enamel hypoplasia.

In individuals with PFEH, most permanent teeth are affected to varying degrees, with second molars having the highest prevalence of PFEH (Table 3). The first molars were not affected by PFEH (Figure 2; Table 3). Although not studied in depth here due to small sample sizes, the low number of deciduous teeth present did not show PFEH, even if the permanent dentition did (Figure 2(c)). PFEH had a similar presentation across individuals and tooth types, with the most prominent examples showing the absence of enamel in large areas of the crown, leaving the dentine protruding (Figures 2 and 3). In some cases, the defects did not affect the occlusal surface, with deep defects observed further down the crown (Figure 3(b)). On some teeth, the defects appeared less severe, making it difficult to distinguish between LEH or PFEH (e.g., Figure 2(c)).

There was no sign of hypo- or hypermineralization in dental tissues in the individual with PFEH analyzed via micro-CT, with MC values similar to those of an individual without PFEH (Table 4). There was also no evidence of extreme pathology/wear (e.g., caries or erosion) further causing demineralization of dental tissue in these areas.

4. Discussion

The results of this study suggest that the severe enamel defects observed in Japanese macaques from the southern regions of Yakushima Island were most likely not the result of a genetic condition (i.e., amelogenesis imperfecta AI) or localized trauma to the developing tooth (i.e., localized enamel hypoplasia). AI typically affects all teeth, including the deciduous and permanent dentition. The PFEH lesions observed on these macaques do not affect the first molars and appear to not affect the deciduous dentition. The lesions observed here are likely not AI because the remaining enamel is sound. If hypo- or hypermineralization of enamel had contributed to the formation of these defects, or else pathology (e.g., caries) or wear (e.g., erosion) was in some way involved, then changes in the MC of the remaining dental tissue would be expected. In studies which estimated MC in hypo- or hypermineralized teeth, and others which assessed the impact of dental pathologies on dental tissue MC, a significant reduction or increase of over 0.25 g/cm^3 has been observed compared to "sound" dental tissue, e.g., [32, 35]. There is no evidence for such MC variation in the macaques analyzed here. We similarly rule out these PFEH being caused by localized enamel defects, as these normally affect a certain surface or tooth and not entire tooth crowns, as is observed here [6, 36].

Yakushima macaques have several distinguishing features from other Japanese macaques, such as a smaller body size and darker coat, as well as a suite of suggested cranial morphological differences with other Japanese macaques [37–39]. Genetic research suggests that these differences are likely due to a few key mutations in their genomes [25, 40]. Yakushima population experienced a significant decline approximately 5000 years ago, which may have been caused by the eruption of the Kikai caldera [40, 41]. Recent human activities including habitat destruction and culling of individuals may have further exacerbated the limited population mobility and increased the likelihood of inbreeding. This is supported by a recent study that found Yakushima macaques show extremely low genetic diversity [42]. Given the uniform nature of these defects across individuals, it is possible that there was a common risk factor for the type of PFEH defect observed, such as a genetic predisposition brought to high frequency due to the documented bottleneck observed in the population. However, the fact that individuals from other locations on the island seem unaffected by PFEH does not support such a scenario, since all Yakushima macaques experienced this bottleneck.

Previous research has documented troop/group sizes of Yakushima macaques ranging from 10 to 50 individuals [43–45]. However, given the extended collection time span in this study, specific individual group affiliations and familial relationships remain unknown. Consequently, assessing the true prevalence of these defects within macaque troops is challenging. Genetic studies present an opportunity to shed light on potential connections among specific populations, such as relatedness among individuals with PFEH, and to uncover the genetic background of these defects. Yakushima macaques may also offer a unique opportunity to study key questions relating to the evolution of the primate dentition, as there are noticeable size differences in their teeth and overall body size compared to other Japanese macaque populations, both on and off the island [26]. Previous research on primates has highlighted the crucial relationship between molar size and recent adaptation/evolution, demonstrating a complex interplay of multiple genes in dental development and accompanying changes in molar cusps with other dental traits [46–48].

Further research is necessary to distinguish normal phenotypic variation from more pathological variation related to physiological stress, especially considering the smaller size of Yakushima macaques compared to other Japanese macaque populations. This also has implications for phylogenetic interpretations, as some of the specimens identified as having PFEH in this study have been used to

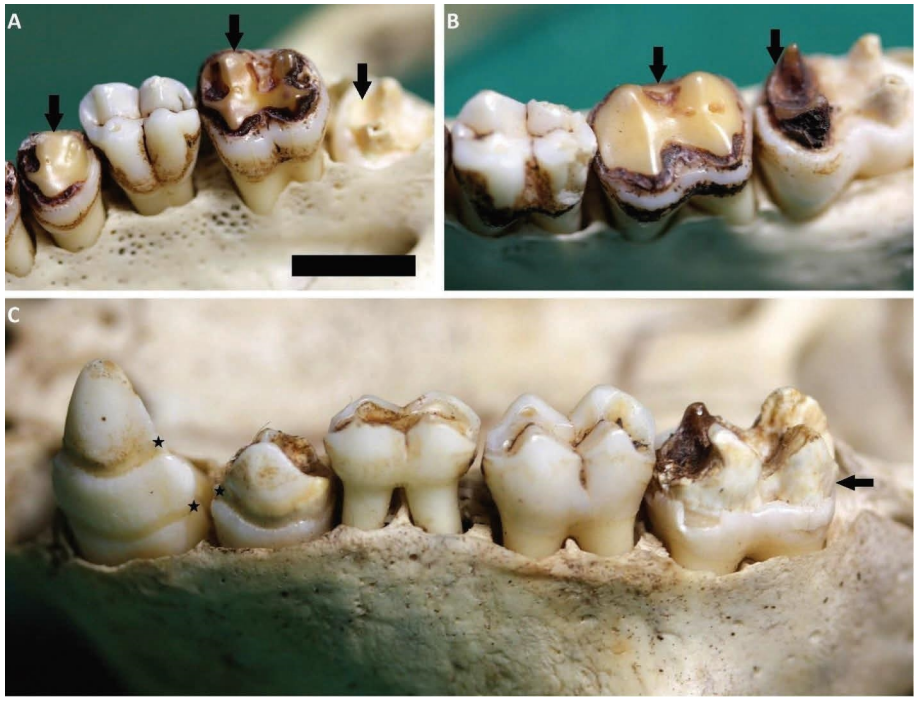

Figure 2: Plane-form enamel hypoplasia (PFEH) on the posterior dentition of Yakushima macaques. (A) Left maxillary posterior teeth (specimen: PRI 2600), showing extensive PFEH on the fourth premolar and second and third molars (black arrows). Note the first molar appears unaffected; (B) mandibular left posterior teeth (specimen: PRI 2600), showing PFEH on both the second and third molars (black arrows). Note the first molar appears unaffected; (C) mandibular left dentition (specimen: PRI 2598), showing PFEH on the second molar (black arrow), and the canine and the third premolar show defects that are best described as PFEH, but could also be termed "severe LEH" (black stars), especially the cervical most defect on the canine. Note the first molar and remaining deciduous molar are unaffected. Scale bar: 1 cm.

Table 2: Enamel hypoplasia prevalence for all permanent teeth ($n = 810$) from 48 individuals, split by defect type.

	Yakushima Island	Other locations
Total observable teeth	330	638
Total teeth with EH	124	34
% teeth with EH	37.6	5.3
LEH	44	27
Localized	12	7
Pitting	0	0
PFEH	68	0
% teeth with PFEH	20.6	0.0
Not observable	74	76
Observable but no EH	206	604

Other locations combine figures for Chiba and Koshima samples. EH, enamel hypoplasia; LEH, linear enamel hypoplasia; localized, localized enamel hypoplasia; pitting, pitting enamel hypoplasia; PFEH, plane-form enamel hypoplasia.

characterize the small size of Yakushima macaques, including smaller dental measurements [26]. This is crucial to consider, since previous studies have shown that variation in molar size is intertwined with prenatal growth rates [49], and smaller tooth size may well be an indicator of environmental stress [46]. The reduction in the first molar size in individuals with PFEH could be related to a combination of pre- and postnatal maternal stress, with greater physiological stress experienced by individuals after birth. A study on rats found that prenatal maternal stress can significantly impact offspring growth, supporting this hypothesis [50]. While limb bones will experience "catch-up" growth later in ontogeny, tooth crowns do not continue to grow after eruption, thereby providing a more accurate reflection of disruptions to early childhood growth [1].

We propose that the PFEH defects likely relate to a severe environmental disturbance experienced by the macaques on the southern part of Yakushima Island, with permanent first molars and deciduous teeth unaffected because they form in utero and therefore would have been maternally buffered from the environmental factors that triggered the PFEH. From the available environmental evidence, macaques living in this region of Yakushima Island experienced an intense human encroachment on their

Table 3: Combined total for each tooth type (including antimeres and maxillary and mandibular teeth), for all Yakushima Island samples combined.

	M3	M2	M1	PM2	PM1	C	I2	I1
Total observable teeth	26	53	59	41	42	41	31	37
Total teeth with EH	9	24	5	15	19	14	14	24
% teeth with EH	34.6	45.3	8.5	36.6	45.2	34.2	45.2	64.9
LEH	3	4	4	3	8	11	7	4
Localized	0	0	1	2	0	0	0	9
PFEH	6	20	0	10	11	3	7	11
% teeth with PFEH	23.1	37.7	0.0	24.4	26.2	7.3	22.6	29.7
Not observable	22	20	19	20	20	10	3	1
No EH	17	29	54	26	23	27	17	13

M, molar; PM, premolar; C, canine; I, incisor. EH, enamel hypoplasia; LEH, linear enamel hypoplasia; localized, localized enamel hypoplasia; PFEH, plane-form enamel hypoplasia.

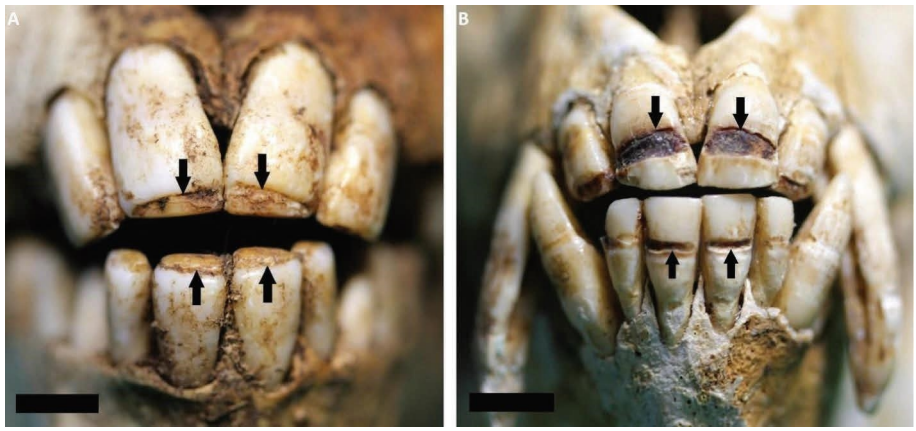

Figure 3: Plane-form enamel hypoplasia (PFEH) in the anterior dentition of Yakushima macaques. (A) Specimen PRI 2604, showing extensive PFEH defects near the incisal edge of the central incisors, leading to protruding dentine (black arrows); (B) specimen PRI 3124 showing PFEH defects in the midcrown region (black arrows). Both scale bars 1 cm.

Table 4: Average mineral concentration (MC) values for enamel and dentine in three Yakushima macaque teeth.

	Enamel MC (g/cm^3)	Dentine MC (g/cm^3)
PRI 3434 M2 (PFEH)	2.42 ± 0.06	1.27 ± 0.08
PRI 3434 M1	2.39 ± 0.05	1.31 ± 0.06
PRI 1730 M2	2.46 ± 0.12	1.35 ± 0.03

A tooth with PFEH (PRI 3434, lower right M2) and without PFEH, but from the same individual that has PFEH on other teeth (PRI 3434, lower right M1), and a tooth with no sign of PFEH on any tooth (PRI 1730, lower left M2).

habitat. Logging operations intensified on Yakushima Island in the 1960s and 1970s, and many areas experienced intensification of agriculture and conifer plantations. By the 1980s, Yakushima Island macaques were regularly crop raiding in many areas, with oranges commonly consumed [51, 52]. This led to an increase in macaque persecution, with trapping and culling occurring regularly [51]. On Yakushima Island, the number of macaques killed or trapped between 1979 and 1988 is estimated to have been over 3,000 [53]. Deforestation, changes in forest composition, and trapping did not occur uniformly across the island, with large areas of national parks retaining much of their primary forest and natural resources. The areas of focus of this study, Yudomari and Nakama, in contrast, experienced substantial changes, with native forest removed and replaced with orange and conifer plantations. We hypothesize that the severe PFEH observed in macaques living in these areas could be related to stress due to the habitat changes brought about by the intensified logging and agriculture. These changes would have led to a reduction in diet diversity, with a likely shift to consumption of cropped plants (especially oranges). In addition, such changes may have led to food becoming scarce at certain times of the year, potentially leading to severe malnutrition. The disturbance to social groups caused by trapping/culling could also have influenced the etiology of PFEH.

Whether habitat loss and/or environmental change are related to the occurrence of PFEH in the Yakushima Island macaques, it is difficult to piece apart the factors directly responsible for the occurrence of the enamel defects. Often, wild Japanese macaque populations will inhabit a mosaic of land types, even within small geographical ranges. Areas

with primary forest, conifer plantations, and naturally regenerated forests will influence and impact the behavior and diet of wild macaques [54]. This means that if enamel defects are observed, understanding the exact cause would be difficult, even if detailed foraging information was available. All of the macaques included in our study were affected by human activities in some way, including Koshima Island macaques being provisioned with food, human activities, and the introduction of nonnative fauna and flora to Chiba on the mainland. Despite this, macaques in these other locations do not show severe PFEH defects or high LEH prevalence compared to other primate species in other geographic regions [7, 20]. This suggests the macaques from Yudomari and Nakama must have undergone severe periods of physiological stress and severe malnutrition, or had enamel that was particularly susceptible to those stresses beyond what has been experienced by other wild primate groups.

However, other natural causes should also be considered. Precipitation is high on Yakushima Island and has been associated with enamel defects in other primate groups [55] and references therein. Similarly, typhoons and other severe weather events regularly affect the island, including during the period of interest [56, 57]. An example of such an event was described by Hanya et al. [43], in which they reported the mass mortality of macaques in 1998 due to natural causes. The authors concluded the mass mortality event was likely related to severe weather (hot and dry) causing low production of fleshy fruits, leading to severe shortage of high-quality foods [43]. The authors also discussed the possibility of intertroop competition and disease, although these seem less likely to result in PFEH. Similarly, changes in home range size and group dynamics could also be linked to increased levels of physiological stress and aggression within macaque populations [54, 58, 59].

Dental fluorosis causing enamel breakdown could also be related to the aetiology of these defects, as evidenced by similar lesions observed in other mammals and marsupials. These defects not only mirror the shape and severity of dental fluorosis-related issues but also align with the unchanged pattern of tooth formation during prenatal development [60–63]. However, the role of dental fluorosis as a primary etiological for such PFEH lesions needs to be further explored. Often other types of defects such as hypoplastic pits and alterations in enamel coloration are associated with dental fluorosis, which was not observed in the sample studied here. Furthermore, the mineral concentration within normal ranges observed in the specimens analyzed via micro-CT does not support dental fluorosis as the aetiology for these lesions.

Since the 1980s, mitigation of the impacts of human occupation on macaques, including plans for sustainable use of forests, has been put in place on Yakushima Island. Nongovernmental organizations (NGOs) have been established to promote conservation and natural history research and to prevent human-wildlife conflicts on the island. Further research into the health and physiological stress of these macaques over time using skeletal indicators such as enamel hypoplasia may offer further insight into the effects of human activity on these populations. Here, we suggest PFEH could be used as a marker of anthropogenic impact, for which there is growing evidence of changes in diet, behavior, and social structures, e.g., [64]. PFEH could also be useful alongside other studies on nutrition and population health, such as previous investigations using urinalysis and fecal analysis [59, 65] and morphological comparisons [66, 67]. Primatological studies have also shown how the feeding behavior and ranging patterns of Yakushima macaques have changed alongside human landscape alterations, both daily and across seasons, e.g., [68]. PFEH is rare in wild primates and archaeological/paleontological hominin samples. Further research into enamel defects may prove useful for assessing the effect of severe environmental events, including human-induced activity, in different primate groups.

5. Conclusions

This study investigated the prevalence and etiology of planeform enamel hypoplasia (PFEH) in wild Japanese macaques from Yakushima Island. Our findings suggest that PFEH defects, which affect a high number of macaques from Yakushima Island, are not attributable to genetic conditions such as amelogenesis imperfecta or localized enamel trauma. PFEH defects seem to be linked to environmental factors and anthropogenic impacts. Furthermore, the study underscores the complexity of factors influencing the formation of dental defects in wild primates, including diet, habitat changes, and genetic predispositions.

Data Availability

The data analyzed for this study are openly available in Dryad (doi: 10.5061/dryad.6t1g1jx46), which also includes the location, year of inclusion in the PRI collection, and sex of each individual studied.

Disclosure

The views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union or the European Research Council. Neither the European Union nor the granting authority can be held responsible for them. This research has been posted to a preprint server [69].

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This research was supported by a Sir Thomas Kay Sidey Postdoctoral Fellowship from the Faculty of Dentistry, University of Otago. This research was additionally facilitated by the European Research Council within the European Union's Horizon Europe (ERC-2021-ADG, Tied2Teeth, project no. 101054659 to LJH). The authors thank the Study Material Committee from the Primate Research Institute (PRI), Kyoto University, for access to their collections, and T. Ito for assistance during data

collection. The research was performed under the Co-operative Research Program of the PRI (2019-C-20). The authors also thank T. Ito and G. Hanya for their helpful comments during the writing of this manuscript. CL acknowledges the University of the Otago Division of Health Sciences for the research and study leave funding and Massey University as the host institution.

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