A New Light on the Evolution and Propagation of Prehistoric Grain Pests: The World’s Oldest Maize Weevils Found in Jomon Potteries, Japan

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Abstract

Three *Sitophilus* species (*S. granarius* L., *S. oryzae* L., and *S. zeamais* Mots.) are closely related based on DNA analysis of their endosymbionts. All are seed parasites of cereal crops and important economic pest species in stored grain. The *Sitophilus* species that currently exist, including these three species, are generally believed to be endemic to Asia’s forested areas, suggesting that the first infestations of stored grain must have taken place near the forested mountains of southwestern Asia. Previous archaeological data and historical records suggest that the three species may have been diffused by the spread of Neolithic agriculture, but this hypothesis has only been established for granary weevils in European and southwestern Asian archaeological records. There was little archeological evidence for grain pests in East Asia before the discovery of maize weevil impressions in Jomon pottery in 2004 using the “impression replica” method. Our research on Jomon agriculture based on seed and insect impressions in pottery continued to seek additional evidence. In 2010, we discovered older weevil impressions in Jomon pottery dating to ca. 10 500 BP. These specimens are the oldest harmful insects in the world discovered at archaeological sites. Our results provide evidence of harmful insects living in the villages from the Earliest Jomon, when no cereals were cultivated. This suggests we must reconsider previous scenarios for the evolution and propagation of grain pest weevils, especially in eastern Asia. Although details of their biology or the foods they infested remain unclear, we hope future interdisciplinary collaborations among geneticists, entomologists, and archaeologists will provide the missing details.

Citation: Obata H, Manabe A, Nakamura N, Onishi T, Senba Y (2011) A New Light on the Evolution and Propagation of Prehistoric Grain Pests: The World’s Oldest Maize Weevils Found in Jomon Potteries, Japan. PLoS ONE 6(3): e14785. doi:10.1371/journal.pone.0014785

Editor: Robert DeSalle, American Museum of Natural History, United States of America

Received June 30, 2010; Accepted March 1, 2011; Published March 29, 2011

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Funding: This research was supported by Grant-in-Aid for Scientific Research on Areas A (subject number: 20242022) by Japan Society for Promotion of Science (http://www.jsps.go.jp/j-grantsinaid/index.html). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Granary (*Sitophilus granarius* L.), rice (*Sitophilus oryzae* L.), and maize (*Sitophilus zeamais* Mots.) weevils, known as “snout weevils”, feed inside rice or barley grains during their larval stage and pupate inside the grains [1]. Rice and maize weevils are widespread in warm regions. In Europe and North America, they are replaced by temperate species such as the granary weevil [2]. The global distribution of these insects occurred relatively recently, and they have continued to spread as a result of worldwide cereal trading during the 20th century.

In archaeological records from Europe, the Mediterranean, and Asia, the oldest granary weevil discovery has been dated to ca. 7000 BP. The granary weevils recovered from many sites in these regions correspond to the diffusion of Neolithic agriculture or Roman cereal trading and transport of soldiers. In contrast, few prehistoric discoveries of the rice weevil and maize weevil had been reported in China and Japan by 2003. In general, the granary weevil and its two sister species are monophyletic species in genus *Sitophilus* and are believed to have originated in Asia [3].

However, many impressions of cereal weevils have been discovered in Jomon pottery in Japan since researchers first applied the “impression replica” method in 2004. This method involves making a model of the original form that created the cavity in a potsherd. Since 2004, the number of impression records has gradually increased. Until 2009, we had collected 37 samples, of which the oldest dated to ca. 4500 BP. However, our recent search for seed or insect impressions at archeological sites on Tanegashima Island in spring 2010 provided many new records, which suggest that the association between maize weevils and humans occurred in the Earliest Jomon (11 500 to 7300 BP), at ca. 10 500 BP. This discovery suggests that researchers must reconsider previous scenarios for the evolution and propagation of grain pest weevils, their adaptation to grain storage systems, and their spread along with prehistoric agriculture, especially in eastern Asia. Our new results suggest that eastern Asian grain pests, including the maize weevil and probably the rice weevil, evolved differently from the granary weevil in Europe.

The goal of this paper is to introduce our new discovery and discuss its preliminary significance for archaeological research in eastern Asia.
Results and Discussion

Discovery of weevil impressions in Jomon potsherds

Greek and Roman records from ca. 2200 BP describe weevils infesting wheat. In China, the oldest record appears in the “Èryà” dictionary, which was published between ca. 2500 to 2200 BP. By this time, weevils were already recognized as storage pests that infested rice. The first Japanese description of maize weevils appears in historical records from ca. 1000 BP [4,5].

Archaeological records from Europe and southwestern Asia reveal that granary weevils had spread along with the diffusion of agriculture and grain trading or transport between ca. 7000 BP and ca. 1900 BP [3]. For example, granary weevils were discovered in a funeral offering of barley to a dead king of the Egyptian Sixth Dynasty (ca. 4300 BP). In China, there is little archaeological evidence of prehistoric grain pests. The only example is of rice weevils infesting barley in a grave from the Han Dynasty (2118 BP) [4,5].

In Japanese archeological records, maize weevil impressions were first discovered in Jomon pottery in 2004. Before then, the oldest specimens (beetle carapaces) came from a ditch deposit at the Ikegami site (ca. 2000 BP). The beetle carapaces of maize

Figure 1. Scanning electron micrograph (SEM) images of impression replicas of maize weevils that had been discovered at Jomon sites by 2009. The 37 weevil impressions were discovered in Jomon pottery, mainly collected on Kyushu, dating to between ca. 4500 and ca. 3000 BP. Based on the diagnostic criteria described in the text, including the size of the specimens, these weevils appear to have been maize weevils (Sitophilus zeamais). Details of each impression replica are provided in Table 1.

doi:10.1371/journal.pone.0014785.g001
### Table 1. Details of the maize weevil impressions that were obtained at Jomon sites by 2009. Numbers correspond to the photographs in Figure 1.

| Part of insect covered by the impression | Site | Sample name | Type of pottery | Shape/part | Phase during the Jomon | Estimated age (BP) |
|-----------------------------------------|------|-------------|-----------------|------------|------------------------|-------------------|
| Dorsal view (missing rostrum, wings, and legs) | Ishinomoto (Kumamoto Pref.) | INM-47 | Amagi | Deep bowl/rim | The end of the Late J. | ca. 3450 |
| Side view (missing legs) | Ishinomoto (Kumamoto Pref.) | 42-29629-1 | Amagi | Deep bowl/rim | The end of the Late J. | ca. 3450 |
| Ventral view (missing rostrum and legs) | Ishinomoto (Kumamoto Pref.) | 45-1697-1 | Goryo | Shallow bowl/rim | The latter half of the Late J. | ca. 3500 |
| Dorsal view (missing legs) | Ishinomoto (Kumamoto Pref.) | 39-SH01-2694 | Koga | Shallow bowl/body | The end of the Late J. | ca. 3400 |
| Ventral view (missing rostrum and legs) | Ishinomoto (Kumamoto Pref.) | 47-SH35-31040-1 | Amagi | Deep bowl/unknown | The end of the Late J. | ca. 3450 |
| Ventral view (missing legs) | Ishinomoto (Kumamoto Pref.) | 47-SX-07-b | Goryo | Shallow bowl/unknown | Last half of the Late J. | ca. 3500 |
| Unknown | Ishinomoto (Kumamoto Pref.) | Unknown | Unknown | Unknown | The Latest J. | ca. 3400 to 3000 |
| Ventral view (missing rostrum and legs) | Kunugibaru (Kagoshima Pref.) | Kunugibaru-1 | Unknown | Deep bowl/rim | The first half of the Latest J. | ca. 3200 to 3000 |
| Side view (missing rostrum and legs) | Kunugibaru (Kagoshima Pref.) | Kunugibaru-2 | Unknown | Deep bowl/rim | The first half of the Latest J. | ca. 3200 to 3000 |
| Side view (missing rostrum and legs) | Kunugibaru (Kagoshima Pref.) | Kunugibaru-3 | Unknown | Deep bowl/rim | The first half of the Latest J. | ca. 3200 to 3000 |
| Ventral view (missing thorax and legs) | Ohnobaru (Nagasaki Pref.) | ONB1010 | Tarozako | Deep bowl/base | The end of the Late J. | ca. 3600 |
| Side view (missing legs) | Higataro (Nagasaki Pref.) | HIG115 | Kurokawa? | Deep bowl/body | The first half of the Latest J. | ca. 3300 |
| Side view (missing legs) | Higataro (Nagasaki Pref.) | 10381-03 | Kurokawa? | Deep bowl/body | The first half of the Latest J. | ca. 3300 |
| Ventral view (missing rostrum and legs) | Gongenwaki (Nagasaki Pref.) | GGW-021 | New Kurokasa | Bowl/body | The middle of the Latest J. | ca. 3000 |
| Side view (missing rostrum and legs) | Ohnobaru (Nagasaki Pref.) | ONB1018 | Tarozako | Deep bowl/body | The latter half of the Late J. | ca. 3600 |
| Ventral view (missing rostrum and legs) | Mimanda (Kumamoto Pref.) | MD0019 | Tarozako | Bowl/base | The middle of the Late J. | ca. 3600 |
| Side view (missing head and legs) | Kaminabe (Kumamoto Pref.) | KNB05 | Amagi | Deep bowl/rim | The end of the Late J. | ca. 3450 |
| Side view (missing head and legs) | Kaminabe (Kumamoto Pref.) | KNB32 | Koga? | Deep bowl/body | The end of the Late J. | ca. 3400 |
| Side view (missing legs) | Kaminabe (Kumamoto Pref.) | KNB34 | Amagi | Deep bowl/body | The end of the Late J. | ca. 3450 |
| Side view (missing rostrum and legs) | Ohbaru D (Fukuoka Pref.) | Ohbaru-D-3 (9265) | Unknown | Deep bowl/unknown | The first half of the Latest J. | ca. 3200 to 3000 |
| Side view (missing rostrum and legs) | Shigetome (Fukuoka Pref.) | Shigetome-1 (8748) | Amagi | Deep bowl/body | The end of the Late J. | ca. 3450 |
| Dorsal view (missing legs) | Toroku shell midden. (Kumamoto Pref.) | TR11 | Kanazaki 3 | Deep bowl/body | The first half of the Late J. | ca. 4000 |
| Dorsal view (missing rostrum and trunk) | Toroku shell midden. (Kumamoto Pref.) | TR21 | Mimanda | Deep bowl/body | The latter half of the Late J. | ca. 3550 |
| Ventral view (a trunk) | Nishibira shell midden (Kumamoto Pref.) | NB02 | Nishibira | Bowl/body | The first half of the Late J. | ca. 3700 |
| Side view (missing legs) | Nishibira shell midden (Kumamoto Pref.) | NB07 | Nishibira? | Bowl/body | The first half of the Late J. | ca. 3700 |
| Dorsal view (missing legs) | Nishibira shell midden (Kumamoto Pref.) | NB08 | Nishibira? | Bowl/body | The first half of the Late J. | ca. 3700 |
| Ventral view (missing legs) | Nishibira shell midden (Kumamoto Pref.) | NB17 | Goryou | Shallow bowl/rim | The latter half of the Late J. | ca. 3500 |
| Dorsal view (missing rostrum and legs) | Kurokamimachi (Kumamoto Pref.) | KKN07 | Unknown | Deep bowl/body | The latter half of the Late J.? | ca. 3600 to 3400 |
Prehistoric Maize Weevils

weevils were also recovered at the Fujiwara Palace site (ca.1190 BP) and the Kiyosu Castle site (ca. 390 BP) [6]. In 2004, maize weevil impressions were discovered (ca. 3500 BP) in the Late Jomon sites in northwestern Kyushu at this time. Furthermore, rice phytoliths have appeared at roughly the same time as the expansion of agriculture, which is known from Japanese archaeobotanical evidence [11,14]. The former hypothesis is accepted by most archaeologists. Evidence from archaeological sites dating to those times (charred seeds, seed impressions, and phytoliths) suggests the cultivation of cereals in the Poaceae (e.g., rice, barley), which were introduced to Kyushu from Korea. The second hypothesis is not supported by archiological or archaeobotanical evidence [16,17]. The earliest rice cultivation on the Ryukyu Islands occurred in ca. 1100 BP and was not introduced from southern China; instead, it was introduced at the time of human immigration from Kyushu into the region [11,14].

If the maize weevil depends on crop cultivation, then it should have appeared at roughly the same time as the expansion of agriculture, which is known from Japanese archaeobotanical records, and indeed, archaeological artifacts suggest increasingly strong cultural relationships between southern Korea and northwestern Kyushu at this time. Furthermore, rice phytoliths have been recovered from the Late Jomon pottery in northwestern Kyushu. The presence of maize weevils therefore suggests the existence of rice or barley cultivation during the Late to the Latest Jomon periods, and that they invaded Japan from Korea, accompanying rice cultivation [11].

New evidence: older maize weevil impressions

We have new evidence that contradicts the original hypothesis. In February 2010, we discovered the oldest impressions of maize

Table 1. Cont.

| Part of insect covered by the impression | Site | Sample name | Type of pottery | Shape/part | Phase during the Jomon | Estimated age (BP) |
|----------------------------------------|------|-------------|-----------------|------------|------------------------|-------------------|
| Dorsal view (missing rostrum and legs)  | Kurokamimachi (Kumamoto Pref.) | KKN08 | Unknown | Deep bowl/body | The latter half of the Late J.? | ca. 3600 to 3400 |
| Dorsal view (a thorax)                 | Kaminabe (Kumamoto Pref.) | KNB22 | Amagi? | Deep bowl/body | The end of the Late J. | ca. 3450 |
| Dorsal view (missing rostrum and legs) | Nakaya (Yamanashi Pref.) | Nky01 | Shimizutennouzan | Deep bowl/body | The first half of the Latest J.? | ca. 3000 |
| Side view (missing rostrum and legs)   | Nakaya (Yamanashi Pref.) | Nky02 | Shimizutennouzan | Deep bowl/body | The first half of the Latest J.? | ca. 3000 |
| Ventral view (missing rostrum and legs) | Mimanda (Kumamoto Pref.) | MMD2054 | Unknown | Deep bowl/body | The latter half of the Late J.? | ca. 3600 to 3400 |
| Ventral view (missing head and legs)   | Ohonobaru (Nagasaki Pref.) | ONB1116 | Unknown | Deep bowl/body | The latter half of the Late J.? | ca. 3600 to 3400 |
| Ventral view (missing rostrum and legs) | Kakiuchi (Kagoshima Pref.) | KKO0019 | Namiki-Nanpukuji | Deep bowl/body | The beginning of the Late J. | ca. 4500 to 4000 |
| Dorsal view (abdomen)                  | Izumi shell midden (Kagoshima Pref.) | KZK0008 | Izumi | Deep bowl/rim | The beginning of the Late J. | ca. 4300 |
| Side view (missing rostrum and legs)   | Nanbaruachibori (Kagoshima Pref.) | NBU0005 | Ichiki | Deep bowl/rim | The beginning of the Late J. | ca. 4200 |

Japanese academic circles on archaeology divides the Jomon Period into six phases as followings. The Incipient Jomon (15000-11500BP), The Earliest Jomon (11500-7300BP), The Early Jomon (7300-5500BP), The Middle Jomon (5500-4500BP), The Late Jomon (4500-3300BP), The Latest Jomon (3300-3000BP).

doi:10.1371/journal.pone.0014785.t001
Weevils, which we obtained from the Earliest Jomon potsherds dated to ca. 10,500 BP from the Sanbonmatsu (SBM) site in Kagoshima Prefecture. The site, which is on a terrace (50 m asl) on the eastern coast of Tanagashima Island, 40 km southeast of the Ohsumi Peninsula, was excavated in 2007 in a project organized by the Nishinoomote City Board of Education. Researchers discovered cultural layers containing many artifacts, mainly from the Earliest Jomon period.

When we examined the potsherds to find seed and other impressions in February 2010, we found two fragments that contained maize weevil impressions. During our second examination, in April 2010, we found five fragments with maize weevil

![Figure 2. Scanning electron micrograph (SEM) images of the maize weevil impression replicas obtained from potsherds from the Sanbonmatsu Site. Maize weevil impressions on pottery (a), pottery section illustrations with rubbings (b), photos of cavities (c), and SEM images of the impression replicas (d,e,f). The white circles indicate the position of the cavities in the pottery. "A" and "B" show the positions of the potsherds with maize weevil impressions in pottery samples SBM0060, SBM0061, SBM0062, SBM0067, and SBM0073. "A" is from the Ohnakahara Site and "B" is from the Kakoinoharu Site in Kagoshima Prefecture. Details are provided in Table 2. doi:10.1371/journal.pone.0014785.g002](image)
impressions. These fragments came from Yoshida-type deep bowls and were ornamented with shells, a popular ornamentation during the first half of the Earliest Jomon period in southern Kyushu [18]. This cluster has 14C dates from ca. 9240 BP to ca. 9330 BP, suggesting that the Yoshida pottery dates to ca. 10 500 cal BP [19].

Adult granary weevils have elongated punctations on their thorax and other body parts, whereas adult rice and maize weevils have round or irregular punctations [20]. Most of the replicas lacked legs and a rostrum, but had round or irregularly shaped punctations, similar to those of maize weevils (Fig. 2, Table 2).

However, two beetles are morphologically similar to maize weevils and should be excluded as possible explanations for the impressions: Diocalandra spp. and Paracythopeus melancholicus Roelofs. Diocalandra spp. are slenderer and longer than maize weevils [21]. The ratio of thorax to elytron length also differs among the three species: 0.898 for Diocalandra spp., 0.500 for P. melancholicus, and 0.757 for weevil impression replica SMB0024, which nearly equals the ratio of 0.776 for S. zeamais (Fig. 3). The side view of weevil impression SMB0024 is most similar to that of S. zeamais (Fig. 3). The diagnostic criterion that distinguishes S. zeamais from P. melancholicus is the elytron end, which is shorter than the abdomen in S. zeamais but covers the full length of abdomen in P. melancholicus (Fig. 3). These criteria can be seen clearly in the elytron end of the other weevil impression replicas (SMB0060, SMB0061, and SMB0062; Fig. 2), which suggest that the weevils from the Sanbonmatsu site were S. zeamais. These are therefore the oldest maize weevil relics in the world.

To confirm this identification, we obtained CT scans of the impressions. These revealed details of the insect’s legs, rostrum end, and antennae that have never previously been seen in weevil impression replicas (Fig. 4). These findings demonstrate the superiority of the CT scans compared with the original replica method for correctly identifying insects.

**Table 2.** Details of the maize weevil impressions from the Sanbonmatsu (SBM) site.

| No.   | Pottery type     | Shape/part       | Part of the weevil in the impression       | Length of impression replicas (mm) | Estimated length of original weevil (mm) |
|-------|------------------|------------------|-------------------------------------------|------------------------------------|-----------------------------------------|
| SBM0011 | Yoshida         | Deep bowl/body   | Ventral view (missing legs)               | 3.69                               | 3.98                                    |
| SBM0024 | Yoshida         | Deep bowl/rim    | Ventral view (missing rostrum and legs)   | 3.32                               | 4.58                                    |
| SBM0060 | Yoshida         | Deep bowl/rim    | Dorsal view (missing rostrum and legs)    | 3.11                               | 4.33                                    |
| SBM0061 | Yoshida         | Deep bowl/rim    | Ventral view (missing legs)               | 4.11                               | 5.41                                    |
| SBM0062 | Yoshida         | Deep bowl/bottom | Dorsal view (missing rostrum)             | 3.45                               | 4.69                                    |
| SBM0067 | Yoshida         | Deep bowl/body   | Ventral view (missing rostrum)            | 3.45                               | 4.69                                    |
| SBM0073 | Yoshida         | Deep bowl/bottom | Dorsal view (missing rostrum)             | 3.58                               | 4.83                                    |

Samples are illustrated in Figure 2. doi:10.1371/journal.pone.0014785.t002

**Significance of maize weevils during the Earliest Jomon**

Plarre [3] describes one accepted scenario for the evolution and propagation of grain pests, as follows. The currently existing *Sitophilus* species, including the three economically important grain pests discussed in this paper (*S. granarius*, *S. oryzae*, and *S. zeamais*), appear to be endemic to the forested areas of Asia. It therefore seems likely that the first infestation of stored grain occurred near the forested mountains of southwestern Asia. If these insects originally infested forest food sources such as acorns, the storage of these foods with cultivated grains would have provided the weevils with an alternative food source, and weevils capable of exploiting this resource would have had an advantage over other weevils, leading to co-evolution that produced weevils that were increas-

- **Prehistoric Maize Weevils**
Figure 3. Diagnostic characteristics used to identify the weevil species. Three species of weevils (Sitophilus zeamais, Diociaandra elongata, and Paracythopeus melancholicus) are distinguishable by the ratio of thorax to elytron length. Another diagnostic criterion that distinguishes S. zeamais from P. melancholicus is the elytron end. Elytron does not cover the full abdomen in S. zeamais but extends the full length of the abdomen in P. melancholicus (bottom row of photographs). doi:10.1371/journal.pone.0014785.g003
Figure 4. CT scan images of the impression of maize weevil SBM0024 from the Sanbonmatsu Site. The CT scan images show details of the insect’s legs, rostrum end, and antennae that were previously unseen in the impression replicas. These findings demonstrate the method’s superiority to the older impression replica method for correctly identifying insects. The lengths in this example are 0.908 mm (rostrum), 1.246 mm (thorax), and 1.934 mm (abdomen). In our experiment, the shrinkage of the mean lengths from the original maize weevils to the impressions they left in potsherd cavities were to 92.16% (rostrum), 91.63% (thorax), and 96.28% (abdomen) of the original length. Therefore, the original lengths would have been 0.985 mm (rostrum), 1.359 mm (thorax), and 2.008 mm (abdomen). These lengths are larger than those of modern reference specimens.
that were reared in cleaned rice grains (mean lengths of rostrum, thorax, and abdomen were 0.903, 1.105, and 1.428 mm, respectively; \( n = 20 \)) and were similar to the size of weevils reared in chestnuts (mean lengths of rostrum, thorax, and abdomen were 1.056, 1.338, and 1.882 mm, respectively; \( n = 20 \)).

doi:10.1371/journal.pone.0014785.g004

source during famines. Indeed, we found charred bamboo seeds at archaeological sites from the Late Jomon to the Ainu Culture period (from ca. 760 BP) on Hokkaido [26]. However, phytolith analysis suggests rapid decreases in bamboo flora in this region (Tanegashima Island) from 11 300 to 7300 BP because of the expansion of evergreen forest [27,28]. On the other hand, acorns and chestnuts are important stored foods and were popular in the Jomon Period [12]. According to the short introduction by Delobel and Grenier [29], cereal weevils are polyphagous, and they successfully reared them in both acorns and chestnuts. Rice weevils prefer larger mature wheat kernels to smaller immature ones [30], and the body size (weight) of the three cereal weevils depends on the size of the plant seeds they infest [29]. Our preliminary experiment showed that adult weevils reared in acorns or chestnuts were about 1.24 times the size of those reared in cleaned rice. Figure 4 and Table 2 shows that the maize weevils at the Sanbonmatsu Site are roughly the same size as those that were reared in acorns and chestnuts. This suggests that bamboo seeds, acorns, or chestnuts might have been the stored foods that became infested by maize weevils during in the Jomon Period. However, additional research is required to provide more data and more accurate data to support that hypothesis.

Do differences in the physiological characteristics and life cycle mean different origins?

Granary weevils and Japanese rice weevils cannot fly, unlike the maize weevil. Granary weevils and probably the Japanese rice weevil also depend strongly upon stored grain. Their complete larval development takes place inside the grains that they infest, and has not been observed in natural reservoirs [31,20]. In other words, they are fully synanthropic grain pests and their spread depends upon the transport of infested grain or a suitable substrate [3]. In contrast, maize weevils shelter in winter under fallen leaves or in the soil [2]. After awakening in the spring, they feed on nectar from flowers. Their home range is within 400 m from human villages with grain storage facilities; thus, dispersal over longer distances requires human assistance (e.g., via grain transport) [26]. The pattern of spending most of their life outdoors is thought to result from an ancient adaptation to surviving on wild plants that produce suitable seeds [32]. Consequently, the maize weevil is clearly different from the granary weevil in terms of its degree of dependence on stored grain and in its life cycle.

Pllatte [3] has suggested that the separation of Sitophilus lineages that led to the current grain pests must have occurred much earlier than previously believed. Therefore, our evidence supports Pllatte’s hypothesis; we believe that weevil pests in the Earliest Jomon in Japan must have evolved from a single common Asian progenitor. Initially, they would not have infested cereal grains (which were not stored at that time) but would instead have infested various kinds of wild plant seeds that were stored by the Jomon people. Our new discovery supports this hypothesis, and should encourage additional research on the ecology and history of maize weevils. In particular, more information is needed about when they began infesting stored food. This will require additional maize weevil specimens from other time periods and regions. No fossils of maize weevils or their ancestors have been discovered, so the origin and history of the taxon remain unclear despite our new evidence. In addition, explicit DNA analysis of modern grain weevils would provide important insights into their phylogenetic relationships.

Materials and Methods

In our previous research, we obtained weevil impression replicas from several sites [11]. Then and in the present study, we began examinations of all potsherds obtained from the Sanbonmatsu Site in Kagoshima Prefecture in 2010. In February and April 2010, we found seven fragments that contained maize weevil impressions.

Weevil models were made from impressions in the clay of the recovered potsherds using the “impression replica” method introduced and adopted by Japanese archaeological researchers in the late 1970s. In this method, researchers inject silicone into a cavity (impression) in the clay, producing a model (replica) of the original form that created the cavity. However, the wet clay used to create a pot contracts as it dries and is fired in a kiln, and this compresses insect specimens. To determine the magnitude of the compression, which can then be used to reconstruct the original size of the trapped insects, we conducted an experiment in which we measured the rostrum, thorax, and abdomen of maize weevils (\( n = 20 \)), then trapped them in wet clay similar in composition to the clay that would have been used to create the potsherds we recovered. Once the clay was dry and had been fired in a kiln, we measured the dimensions of the impression replicas, and calculated the shrinkage ratio as the impression replica size divided by the original size.

To allow a comparison of the impression replicas with modern grain weevils, we obtained data on modern weevils from my collection in Kumamoto City. The maize weevil resembles the rice weevil, but adult rice weevils are 2.1 to 2.9 mm long (mean, 2.3 mm), versus 2.3 to 3.5 mm (mean, 2.8 mm) for adult maize weevils [33].

To understand the developmental biology of the weevils, which would provide clues to their potential anthropogenic food sources, we reared maize weevils on many different foods. In total, we reared the insects on eight foods: rice, wheat, barley, adzuki bean, soybean, acorn, chestnut, and hemp. We found that the weevils could survive on rice, wheat, barley, acorn, and chestnut seeds. For weevils that survived to adulthood in rice, acorn, and chestnut, we measured their total length (\( n = 180 \)) to determine whether the size of the food source affected their maturation and growth.

Although impression replicas from potsherds are important sources of information, they are difficult to examine because of their small size. To test whether modern technology could improve our ability to extract information from the impression replicas, we obtained scanning electron microscope (SEM) micrographs of the impression replicas and CT scans of the impressions. This work was performed at the Archaeological Center of Kumamoto University and the X-Earth Center of Kumamoto University, respectively, following each lab’s standard protocols for scans of small objects.

Acknowledgments

We thank Dr. Katsuru Morimoto, an honorary professor at Kyushu University, for useful information about weevils in Japan, and Mr. Junichiro Okita, curator of the Board of Education, Nishinoomote City, for his assistance with our research. We also thank Prof. Yuzo Obara and Prof.
Author Contributions

Wrote the paper: HO. Performed lab work: AM. Took SEM Photos: AM. Co-Searcher of the findings: AM NN TO YS.

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