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Ancient origin and recent range expansion of the maize weevil *Sitophilus zeamais*, and its genealogical relationship to the rice weevil *S. oryzae*

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Abstract

Archeological records attest the early association of *Sitophilus* with stored cereals from the beginning of agriculture on Asia. The maize weevil (*Sitophilus zeamais*) became particularly damaging to maize, a cereal crop domesticated on Mesoamerica. We investigated the late evolutionary history of the maize weevil to gain insights on its origin, timing of association with maize, and genealogical relationship to the almost morphologically indistinguishable rice weevil (*Sitophilus oryzae*). Two mitochondrial genes (cytochrome oxidase subunit I and cytochrome oxidase subunit II) and the nuclear ribosomal gene region were partially sequenced. Analyses showed that the maize weevil shared no haplotypes with the rice weevil; instead, each species exhibited distinct mitogroups and ribogroups. The two weevil species likely split about 8.7 million years ago (95% highest posterior density: 4.0–15.0). Microsatellite data analyses sorted the 309 specimens from 15 populations of the maize weevil into three genotypic groups, which displayed low genetic differentiation and widespread occurrence worldwide. The maize weevil and the rice weevil are each a distinct species; both of which emerged prior to the onset of agriculture. The maize–maize weevil association took place after maize became widespread as a global crop. The maize weevil populations lack spatial genetic structure at the regional, continental, and intercontinental scales.

Keywords: cereal, maize weevil, phylogeography, population genetics, SSR markers, stored grain pest

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Introduction

The independent emergence of agriculture took place in widely dispersed regions of the world between 10,000 and 6000 years before present (yBP) and allowed for the domestication of important crops, such as cereals and pulses in the Fertile Crescent (Lev-Yadun *et al.*, 2000; Brown *et al.*, 2009), rice in Southeast Asia (Kealhofer & Piperno, 1994), cereals in Northern India (Fuller *et al.*, 2007), and maize in Mesoamerica (Iriarte *et al.*, 2004; Piperno *et al.*, 2009). Insects and insect remains, especially of stored grain insects, are important sources of information for human history in a variety of contexts. They are frequently present in archeological sites and have been important in tracing past urban environments,

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grain origin and likely routes of grain trade, and history of storage (Solomon, 1965; Buckland, 1981; Oliveira *et al.*, 2013).

Historically, several species of pest insects have been associated with stored grains and grain products. Three species of *Sitophilus* Schönherr, 1838 (Coleoptera: Curculionidae) are amongst the most damaging pest insects of stored cereals, leading to severe losses worldwide: the granary weevil *Sitophilus granarius* (L., 1758), the rice weevil *S. oryzae* (L., 1763), and the maize weevil *S. zeamais* (Motschulsky, 1855) (Longstaff, 1981; Throne & Cline, 1989; Levinson & Levinson, 1994; Plarre, 2010). The granary weevil is a flightless and fully synanthropic species that is adapted to artificial grain stores (Plarre, 2010); this species is particularly abundant in archeological sites, but is absent from natural reservoirs (Solomon, 1965; Buckland, 1981; Levinson & Levinson, 1994; Panagiotakopulu, 2001). The rice weevil is a scarce flier and recorded outdoors, it occasionally occurs in cereals in the field; the maize weevil is a more intensely flying species and frequently infests maize in the field before harvest (Longstaff, 1981; Throne & Cline, 1989; Corrêa *et al.*, 2013). The grain weevils (i.e., the granary, rice, and maize weevils) seem to exhibit ecological preferences involving climatic gradients: the granary weevil in temperate climates, rice weevil in more subtropical areas, and the maize weevil in warmer climates (Longstaff, 1981; Throne & Cline, 1989; Levinson & Levinson, 1994; Plarre, 2010; Corrêa *et al.*, 2013). Although host specificity is not strict (Haines, 1981; Throne & Cline, 1991) host preferences seem to distinguish the grain weevils to a certain extent. The maize weevil shows preference toward larger grains (e.g., maize), while both the rice weevil and the granary weevil favor small grains (e.g., wheat and rye) (Haines, 1981; Throne & Cline, 1991; Corrêa *et al.*, 2013). The rice weevil is almost morphologically indistinguishable from the maize weevil, except for differences on both male and female genitalia, which are difficult to dissect and examine – particularly for females (Kuschel, 1961; Halstead, 1963; Hidayat *et al.*, 1996). The rice weevil and the maize weevil are regarded as 'sibling species'; they have been considered two races of the same species in the past and later recognized as distinct species (Richards, 1944; Kiritani, 1956; Halstead, 1963; Hidayat *et al.*, 1996).

The likely geographic origin of the genus *Sitophilus* is not yet known. Several species of *Sitophilus* feed on seeds of wild trees from forest areas around the Himalayas and the Indian subcontinent (Plarre, 2010). The tamarind weevil *S. linearis* (Herbst, 1797), for example, is native to India and has dispersed to all areas where tamarind is grown (Cotton, 1920). The grain weevils most likely originated from acorn-feeding individuals that became associated with natural stores or reservoirs of acorns associated with bird and rodent nests (Levinson & Levinson, 1994; Plarre, 2010). In Asia, the onset of agriculture during the Neolithic period (about 10,000 yBP) may have provided the grain weevils with the opportunity to expand their territories and change their behavior to feed on stored grains. Weevil fossil impressions (dubiously recognized as maize weevil) from Jomon pottery in Japan (ca. 10,500 yBP) (Obata *et al.*, 2011) and fossils of the granary weevil from Israel and Europe (ca. 7000 yBP) (Buckland, 1981; Panagiotakopulu, 2001) and Egypt (ca. 4300 yBP) (Solomon, 1965; Buckland, 1981; Panagiotakopulu, 2001) attest the early association of these pest insects with stored cereals. The subsequent expansion of cereal crops, initially on Asia and later on toward other continents, likely led to the dispersal of grain weevils across Asia at first and subsequently across Europe, Africa, and the Americas (Plarre, 2010; Obata *et al.*, 2011).

Despite the economic, archeological, and historical importance of *Sitophilus*, the origin and diffusion of the grain weevils remain controversial – particularly the maize weevil. The ecological differences among the grain weevils together with the fossil records suggest two plausible hypotheses to account for the origin of the maize weevil: (a) a common, ancient origin of the maize weevil on Asia alongside the granary and rice weevils, or alternatively (b) a derived origin from a strain of the rice weevil – its 'sibling species' – that recently adapted to maize as the cultivation of this cereal became widespread as a result of the Columbian Exchange (Crosby, 2003). Moreover, the unprecedented speed with which insect pests can disperse worldwide owing to modern agricultural settings and human-mediated transport of infested grains likely allows for adaptive gene combinations (e.g., insecticide resistance) to become widespread rapidly. Thus, it is of phytosanitary concern to uncover the extent of which the maize weevil retains population structure at both the regional and continental levels.

Phylogeography can shed light onto the evolutionary history of the maize weevil, therefore disentangling both its genealogical relationship to the rice weevil and the timing of its association with maize. Herein we explored the late evolutionary history of the maize weevil using mitochondrial and nuclear gene sequences and microsatellite markers from populations sampled from around the world. We addressed the following four questions: (a) Did the maize weevil emerge recently from the rice weevil? That is, the rice weevil is the actual ancestor of the maize weevil owing to an agriculturally driven speciation event. (b) Alternatively, did both the maize weevil and the rice weevil share a most recent common ancestor that predates the onset of agriculture? That is, the two weevil species emerged from a common ancestor and their speciation was not an agriculturally driven event. (c) Which are the current genetic structure and levels of gene flow amongst maize weevil populations, considering either the regional, continental, or intercontinental levels? (d) What are the implications of these findings to justify phytosanitary concerns?

Material and methods

Sampling

Sampling of the maize weevil took place in 53 localities spread throughout 15 countries; while the rice weevil was obtained from 20 localities spread throughout 13 countries (fig. 1, tables S1 and S2 in the supplementary material). Specimens ($n \geq 20$) from each location were fixed in 95% ethanol and kept at −20°C until subsequent use. Species identification was carried out inspecting the insect genitalia in the laboratory (Halstead, 1963); additionally, identification was confirmed using species-specific molecular tools (Corrêa *et al.*, 2013).

DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

Total genomic DNA was extracted following Clark *et al.* (2001). For the maize weevil, the PCR amplified fragments of both the mitochondrial cytochrome oxidase subunit I (COI) gene and the cytochrome oxidase subunit II (COII) gene following protocols described previously (Corrêa *et al.*, 2013, 2014). For the rice weevil, amplification of the COI fragment was carried out with a primer pair described previously by Corrêa *et al.* (2013), while the amplification of the COII

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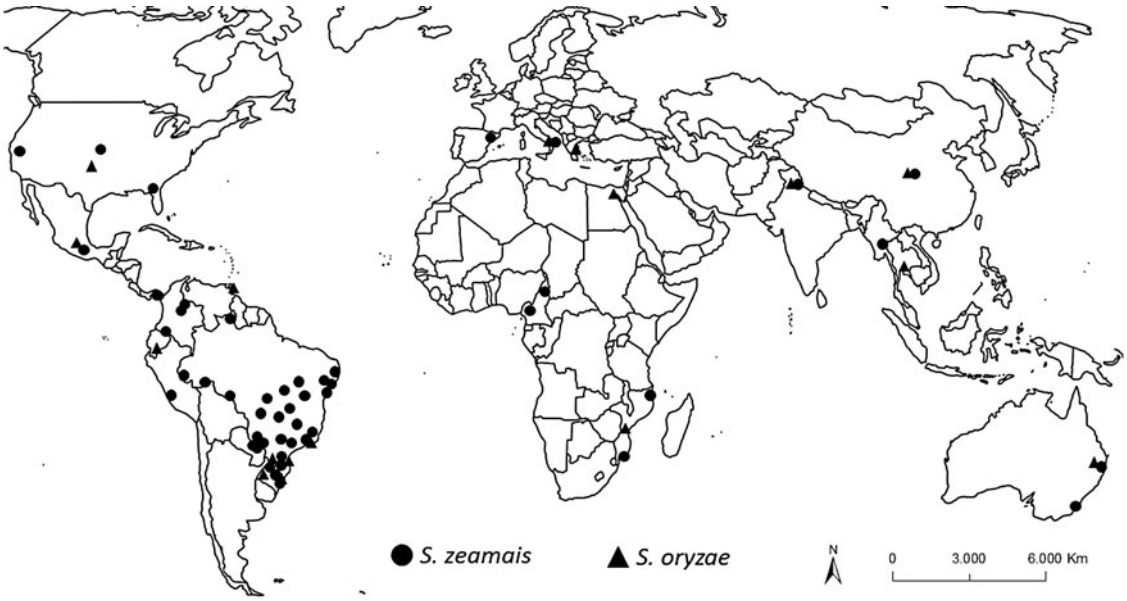

Fig. 1. Geographic distribution and sampling localities of the 53 populations of maize weevil (*Sitophilus zeamais*) and 20 populations of rice weevil (*Sitophilus oryzae*) used in this study (for details, tables S1 and S2 in the supplementary material).

fragment was carried using a pair of primers designed with information from public databases (GenBank accession no. AY014881) and the Primer 3 software (Whitehead Institute for Biomedical Research, Cambridge, MA, USA): forward primer (5′–TTTCTTCAAGATAGAGCCTCACC–3′) and reverse primer (5′–GCTCCGCAAATTTCAGAACA–3′). The nuclear internal transcribed spacer (ITS) region (ITS1–5.8S gene–ITS2) from the ribosomal DNA gene (nrDNA) was amplified using the universal ITS1 primer (White *et al.*, 1990) in combination with a species-specific ITS2 reverse primer (Peng *et al.*, 2003): either 5′–CGATTGTACGAGACGGGCA–3′ (for the maize weevil) or 5′–CCGTTTAAACGATTTCATCC–3′ (for the rice weevil). For the amplification of the ITS region, we used the PCR conditions mentioned previously by Corrêa *et al.* (2014), except that we increased the elongation time to 2.0 min. Amplicons were sequenced using the DNA sequencing services of Macrogen Inc., South Korea (www.macrogen.com).

Assembly of sequence datasets

Sequences were imported into the program Sequencer ver. 4.8 (Gene Codes Corp.) for alignment and editing. For each mitochondrial gene (COI and COII), we obtained 360 sequences (maize weevil, $n = 261$; rice weevil, $n = 99$). Sequence for the ITS region, we obtained 96 sequences of the maize weevil and nine sequences for the rice weevil. After all of the sequences were aligned, their ends were trimmed to eliminate fragments that could not be obtained for all sequences. The alignments had 806, 551, and 1230 bp, for COI, COII, and ITS, respectively. Some sequences of ITS1 region of the maize weevil harbored a 3-bp indel (insertion/deletion event), which consisted of a microsatellite (ACG_4 *versus* ACG_5). Sequences obtained for this study were deposited in GenBank (COI: KJ397732–KJ397813, KX190215–KX190493; COII: KJ397814-KJ397895, KX190494–KX190772; ITS: KX190064–KX190214).

The presence of numts (nuclear paralogs of mitochondrial origin (Lopez *et al.*, 1994); amongst COI and COII sequences was inspected with the help of MEGA ver. 5 (Tamura *et al.*, 2011). The following signatures of numts were searched: (a) *indels* that introduce frameshifts, (b) out-of-place inframe stop codons that lead to premature termination of protein translation, and (c) lack of codon position substitution bias toward the third position that lead to a higher rate of non-synonymous mutations. The presence of signatures (a) and (b) was enough to consider a given sequence as a numt; signature (c) was used to confirm the numt status of the sequence. The sole presence of signature (c) was not used to declare a numt.

Subsequently, we assembled three datasets. Dataset A ($n = 360$; 1357 bp) contained the sequences of both COI and COII concatenated. Dataset B ($n = 96$; 1230 bp) contained following regions: ITS1 (763 bp, partial); 5.8S rRNA (63 bp, complete); and ITS2 (399 bp, partial). Dataset C ($n = 4$; 806 bp) contained the most prevalent haplotype of COI from each of the two weevil species (GenBank accessions KX190227 and KX190400, respectively), supplemented with sequences available from the GenBank for the granary weevil (AY131101) and the tamarind weevil (AY131102).

Demographic statistics

Measures of nucleotide diversity were carried out according to the geographical origin of the specimens: Americas, Europe–Africa, and Asia–Australia. We assembled species-specific sub-datasets from Dataset A. Haplotype diversity and nucleotide diversity parameters were calculated using DnaSP ver. 5 (Librado & Rozas, 2009). Both Tajima's D and Fu's Fs tests of selective neutrality were performed in Arlequin 3.1. (Excoffier *et al.*, 2006). The neutrality tests were tested for significance by generating 1000 random samples using coalescent simulations. The 'Infer from distance matrix' option for 'Haplotype definition' was activated, following the

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recommendation in the Arlequin manual; Fu's Fs statistics were considered as significant at 5% if $P < 0.02$. Analysis of molecular variance (AMOVA) was performed using the Arlequin 3.1 with parametric bootstrapping (1000 replicates) and significance at the 5% level (Excoffier *et al.*, 1992).

Genealogical inferences

Haplotype definition and genealogical relationships among haplotypes in datasets A and B were obtained independently, using the median joining (MJ) method (Bandelt *et al.*, 1999) as implemented in Network 4.5.0.2 (Fluxus Technology Ltd.). We allowed network analyses to infer genetic connections, without taken into consideration that the sequences were obtained from distinct weevil species. We obtained two networks, one for each dataset. Ambiguities in each haplotype network were resolved using the criteria of coalescent theory (haplotype frequency) and population geography (geographical proximity) (Crandall & Templeton, 1993).

Divergence dating

The times of divergence among species of weevil were estimated using Beast v1.7.5. (Drummond *et al.*, 2012). Beauty version 1.7.5 was fed with sequence information from dataset C to generate an XML input file (available upon request). The XML file implemented the following steps: the General Time Reversible model plus invariant sites (GTR + I), which the Akaike Information Criterion (Akaike, 1974) had selected as the best fit model amongst 24 models of molecular evolution using MrModeltest v. 2.3. (Nylander, 2004); the strict molecular clock assumption; the Yule speciation process model selected for tree prior; and a mean substitution rate of 0.0177 substitutions per site per million year (My) for the COI gene (Papadopoulou *et al.*, 2010), which correspond to 3.54% pairwise divergence per My. The XML file assumed that the mutation rate of the COI gene followed a normal distribution (mean = 0.0177; SD = 0.001), matching the 95% interval [0.0157, 0.196]. This analysis allowed us to estimate the age of the most recent common ancestor (tMRCA) for the maize weevil and the rice weevil using the highest known mutation rate for COI gene in beetles (Papadopoulou *et al.*, 2010). The analysis was run for 500 million generations, samples taken every 1 million generation, with three independent replications. Results of each replication were checked for sampling, mixing, and convergence of the Markov chains to a stationary distribution using Tracer v.1.5 (Drummond *et al.*, 2012). Subsequently, we pooled the results of the three replications using Tracer. These settings ensured that both model parameters and time estimates were sampled adequately, as the effective sample size (ESS) values were above 200 for all statistics in Tracer. Means and 95% highest posterior density (HPD) intervals were determined in Tracer.

Microsatellite-based analyses in maize weevil

Ten microsatellite markers (Barat *et al.*, 2012) were applied to 309 specimens, which had been sampled from 15 populations: California (USA), Mexico (MEX), Panama (PAN), Colombia (COL), Brazil (seven locations, BR1 to BR7), Mozambique (MOZ), China (CHI), India (IND), and Thailand (THA). Amplifications were performed in multiplex sets using two triplex sets (loci 3H6-1D10-3G7 and 1A1-1B1-1G7) and two duplex sets (3A11-3G1 and 1E1-1B10). Reactions were performed in a final volume of 13 µl containing template DNA (50 ng), 1× PCR buffer (10 mM Tris–HCl pH 8.4, 50 mM KCl, 1% Triton X-100, 1.5 mM $MgCl_2$), $MgCl_2$ (3.0 mM), dNTPs (50 µM each), forward and reverse primers (0.3 mM each), and 1 U *Taq* DNA polymerase (Phoneutria). The amplification conditions were 4 min at 94°C for the initial denaturation followed by 35 cycles of 30 s denaturation at 94°C, 45 s annealing at 55°C and 1 min elongation at 72°C, with a final elongation at 72°C for 20 min. The amplicons were sized on an ABI PRISM 3100xl DNA Analyser using the GeneScan 500 ROX Size Standard (Applied Biosystems, Foster City, CA, USA)

Hardy–Weinberg equilibrium and linkage disequilibrium were estimated on each of the 15 populations using the F_{STAT} software (Goudet, 2002) and the frequency of null alleles was estimated using the applicative MICRO-CHECKER ver. 2.2.3. (Van Oosterhout *et al.*, 2004). Allelic frequencies, private alleles (A_{PRIV}), expected/observed heterozygozity and coefficient of fixation (F) were calculated using the software GDA 1.1 (Genetic Data Analysis) (Lewis & Zaykin, 2001). AMOVA was performed with Arlequin 3.1 (Excoffier *et al.*, 2006) for two hierarchical levels (among and within populations) using option R_{ST}; the parameters of molecular variance were tested using 1000 permutations. The genetic differentiation among populations was estimated using the parameters F_{IS}, F_{IT}, and F_{ST} (Weir & Cockerham, 1984) G_{ST} (Nei, 1973) and R_{ST} (Slatkin, 1995) with the software F_{STAT} ver. 2.9.3.2 (Goudet, 1995), and the significance was tested following 1000 permutations with 95% confidence interval.

The Bayesian clustering approach of Structure ver. 2.3.4 (Pritchard *et al.*, 2000) inferred the number of genetic groups of the maize weevil using the Monte Carlo Markov Chain (MCMC) approach. The following parameters were enforced: allele frequencies independent; use no admixture model; no location priors were used. We set runs with a burn-in period of 250,000 steps followed by 750,000 steps, with 20 independent replications for every K. As suggested by Evanno *et al.* (2005) the K was set from 1 to 18, which is from one to the number of sampled populations (15) plus three. The best K was determined according to the ΔK method of Evanno *et al.* (2005) calculated with the program Structure Harvester (Earl & vonHoldt, 2012). We converged the data of the 20 replications in the best K with the software Clumpp (Jakobsson & Rosenberg, 2007).

Results

Demographic statistics

Signatures of numts were absent both from COI and COII sequences; therefore, we kept the sequences for subsequent analyses. Measures of nucleotide diversity and neutrality test statistics were carried out on each species separately given that these are intraspecific measures.

For the maize weevil, there were 18 haplotypes among the 261 sequences of the subdataset of mitochondrial genes (COI + COII) and six haplotypes among the 96 sequences of the subdataset of ITS sequences (table 1). There were instances in which we found intraindividual polymorphism for ITS, which were restricted to a single site across the dataset. We created two alternative ITS sequences for each specimen that exhibited the ambiguity. Haplotype diversity (Hd) for sequences of mitochondrial origin reached values that were similar to those found for sequences of nuclear origin (about 0.72). The sample size was much smaller in 'Asia-Australia' (COI + COII, $n = 31$; ITS, $n = 11$) than in 'Americas' (COI + COII, $n = 200$; ITS,

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Table 1. Measures of genetic diversity and neutrality test statistics for *Sitophilus zeamais*, based on two concatenated mitochondrial genes (COI and COII) and the nuclear internal transcribed spacer (ITS) region.

Geographical origin	Sampled size (no. of haplotype)	Haplotype diversity (Hd)	Nucleotide diversity (pi)	Tajima's D (*p* value)	Fu's Fs (*p* value)
Concatenated set (COI + COII)					
Pooled	261 (18)	0.720	0.0012	−0.892 (0.21)	−6.371 (0.03)
Americas	200 (13)	0.710	0.0005	−0.684 (0.27)	−2.467 (0.20)
Europe–Africa	30 (4)	0.586	0.0006	−0.240 (0.41)	0.186 (0.55)
Asia–Australia	31 (7)	0.712	0.0009	−0.504 (0.34)	−1.949 (0.09)
ITS					
Pooled	96 (6)	0.719	0.0007	1.375 (0.89)	−0.128 (0.48)
Americas	73 (4)	0.717	0.0007	2.337 (0.99)	1.424 (0.78)
Europe–Africa	15 (4)	0.743	0.0008	1.931 (0.98)	−0.193 (0.41)
AsiaAustralia	11 (6)	0.743	0.0007	0.467 (0.71)	−2.333 (0.02)

$n = 73$); however, both of these groups exhibited similar values of nucleotide diversity. These results indicated that the small sample size of 'Asia–Australia' was able to capture a relatively large amount of polymorphism. For the rice weevil, there were ten haplotypes among the 99 sequences of the mitochondrial genes (table 2); however, no polymorphisms were found among the nine sequences of the ITS region. Once again, measures of diversity in 'Asia–Australia' reached high values, even though this group had a small sample size.

Tests of selective neutrality in both weevil species showed non-significant values for Tajima's D ($P > 0.05$) and Fu's Fs ($P > 0.05$) for most geographic groups (tables 1 and 2). Such non-significant values are consistent with COI, COII, and ITS sequences harboring randomly evolving mutations (neutrally evolving DNA). The only exception was the significant negative values of Fu's Fs in 'Asia–Australia' of the maize weevil (table 1). Such significant negative values of Fu's Fs indicated that the populations of the maize weevil in 'Asia–Australia' harbored an excess of low-frequency polymorphism and suggested either population expansion or purifying selection.

The results of AMOVA suggested that both weevil species lacked spatial genetic structure at regional, continental, and intercontinental spatial scales (tables S3–S5 in the supplementary material). Differences within groups accounted for most of the genetic variances for the maize weevil (mtDNA 86.6%, $P < 0.001$, table S3 in the supplementary material; ITS 99.6%, $P = 0.33$, table S4 in the supplementary material) and the rice weevil (mtDNA 86.2%, $P < 0.001$, table S5 in the supplementary material).

Haplotype networks

Genealogical relationships among haplotypes of the maize weevil and haplotypes of the rice weevil were assessed using MJ networks, which were constructed for the concatenated mitochondrial genes (fig. 2) and ITS region (fig. 3), separately.

There were 28 haplotypes for the concatenated set of the mitochondrial genes (fig. 2). Amongst the 28 haplotypes, 18 were recovered from specimens of the maize weevil, while the remaining ten haplotypes were from specimens of the rice weevil. For the maize weevil, haplotype 1 was recovered from 47.5% of the specimens and was the most widely distributed haplotype, with worldwide occurrence. For the rice weevil, the most frequent haplotype (haplotype 26; 48%) was also the most widely distributed haplotype. From both weevil species, low-frequency haplotypes were geographically restricted. The topology of the mitochondrial network showed a clear differentiation between the two weevil species. Most notably, there was no haplotype sharing between the two species. Each species exhibited its own distinct mitogroup (i.e., a group of closely related mitochondrial haplotypes). There were 167 mutational steps (from haplotype 9 to either haplotype 20 or 23) between the two mitogroups.

When compared to the mitochondrial network, the ITS network exhibited much less haplotype diversity. There were only seven haplotypes: sequences of the maize weevil displayed six haplotypes, while sequences of the rice weevil exhibited a single haplotype (fig. 3). There was no haplotype sharing between the two species of weevils; thus, each weevil species exhibited its own ribogroup (i.e., a group of closely related ITS haplotypes). The small sample size ($n = 9$) may account for the presence of a single ITS haplotype in the rice weevil ribogroup. There were ambiguities that we could not resolve using the criteria of Crandall & Templeton, (1993), therefore, we maintained these ambiguities in the network – showed as tip haplotypes having more than one connection to central haplotypes (fig. 3). Haplotypes A, B, C, and D (on the ribogroup of the maize weevil) and haplotype G (on the ribogroup of the rice weevil) displayed the highest frequencies and were distributed over a vast geographical area. Haplotypes E and F were singletons (i.e., they occurred only once in our dataset); these two haplotypes were obtained from a single specimen of the maize weevil from Thailand, which exhibited intragenomic polymorphism for ITS. Overall, there were no clear relationship between network architecture and geographic origin of the specimen (figs 2 and 3). In both networks, there was a tendency of finding haplotypes of medium to high frequency with origins within two to three ranges, while most of the haplotypes of low frequency were found within a single range.

Estimated date of divergence

The Beast analysis estimated the time of divergence between the maize weevil and the rice weevil. The results suggested that the two weevil species split from the tMRCA about 8.7 My ago (95% HPD: 4.0–15.0).

Microsatellite-based structure in maize weevil

Our analyses used eight out of the ten microsatellite loci we tested. Locus 1B10 was monomorphic, while locus 1B1

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Table 2. Measures of genetic diversity and neutrality test statistics for *Sitophilus oryzae*, based on two concatenated mitochondrial genes (COI and COII).

Geographical origin	Sampled size (no. of haplotype)	Haplotype diversity (Hd)	Nucleotide diversity (pi)	Tajima's D (*p* value)	Fu's Fs (*p* value)
Concatenated set (COI + COII)					
Pooled	99 (10)	0.732	0.0018	0.509 (=0.74)	0.176 (=0.57)
Americas	47 (4)	0.507	0.0013	1.553 (=0.93)	2.836 (=0.91)
Europe–Africa	25 (3)	0.680	0.0020	2.166 (=0.98)	5.001 (=0.97)
Asia–Australia	27 (7)	0.795	0.0020	0.522 (=0.74)	0.331 (=0.60)

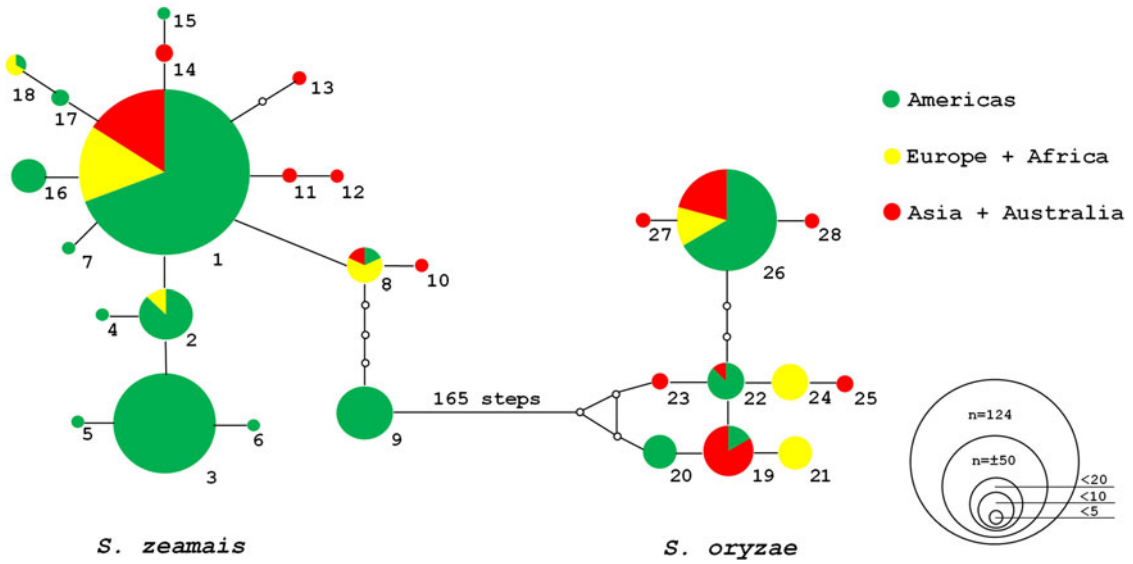

Fig. 2. Median-joining network for the mitochondrial haplotypes of maize weevil (*Sitophilus zeamais*) and rice weevil (*Sitophilus oryzae*). The network was based on a dataset containing two gene regions concatenated: the cytochrome oxidase subunit I (COI) gene and the cytochrome oxidase subunit II (COII) gene. A circle represents a given haplotype (coded with numbers); circle size is proportional to the relative frequencies. Numbers of mutational steps are indicated with small (empty) circles when more than one (unless indicated otherwise). Color codes according to the geographic origin of the sequence.

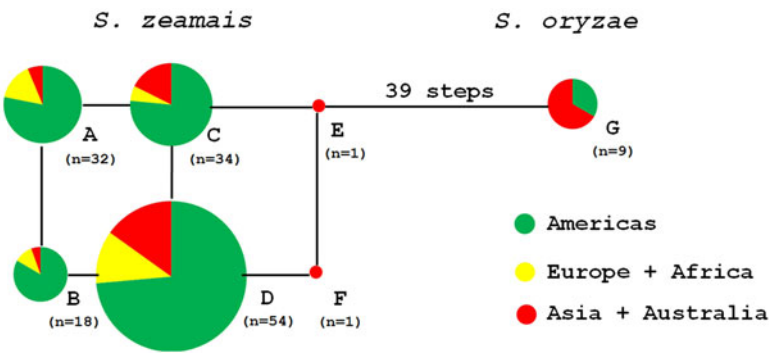

Fig. 3. Median-joining network for the ITS haplotypes of maize weevil (*Sitophilus zeamais*) and rice weevil (*Sitophilus oryzae*). A circle represents a given haplotype (coded with letters); circle size is proportional to the relative frequencies. Numbers of mutational steps are indicated with small (empty) circles when more than one (unless indicated otherwise). Color codes according to the geographic origin of the sequence.

produced bands from a very low number of specimens; both loci were removed from subsequent analyses. After Bonferroni`s correction, no linkage disequilibrium was observed. The mean number of alleles was 3.7, ranging from 3.0 (population BR2) to 4.9 (population BR7); average heterozigosity (expected/observed) was 0.58/0.44 (table 3, table S6 in the supplementary material). The observed heterozygosity in all maize weevil populations was low, with inbreeding

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Table 3. Estimates of genetic diversity of *Sitophilus zeamais* based on eight microsatellite loci (see table S6 in the supplementary material for raw data), with mean number of individuals with locus successfully amplified (*n*), average number of alleles (*A*/locus), number of private alleles (A_{priv}), allelic richness (AR), expected heterozygosity (He), observed heterozygosity (Ho), and inbreeding coefficient (*F*).

Population	*n*	*A*/locus	$A_{priv.}$	*AR*	H_E	H_O	*F*
Richvale, CA, USA (USA)	20.87	3.50	2	2.13	0.54	0.48	0.11
Tlatizapán, Mexico (MEX)	20.00	3.29	1	2.06	0.58	0.46	0.21
Ciudad Panama, Panama (PAN)	19.38	3.75	0	2.16	0.54	0.35	0.37
La Hormiga, Colombia (COL)	20.00	3.25	1	1.91	0.46	0.33	0.31
Rio Branco, AC, Brazil (BR1)	6.50	3.13	1	2.28	0.53	0.38	0.31
Boa Vista, RR, Brazil (BR2)	9.50	3.00	0	2.15	0.62	0.54	0.14
Colônia do Piauí, PI, Brazil (BR3)	22.25	3.62	0	2.20	0.58	0.54	0.06
Palmas, TO, Brazil (BR4)	20.50	4.25	2	2.26	0.60	0.48	0.20
Planaltina, GO, Brazil (BR5)	21.88	3.75	0	2.27	0.60	0.34	0.45
Viçosa, MG, Brazil (BR6)	20.25	4.25	2	2.35	0.64	0.45	0.31
Eldorado do Sul, RS, Brazil (BR7)	21.13	4.88	3	2.33	0.62	0.43	0.31
Maputo, MP, Mozambique (MOZ)	21.25	3.38	3	1.99	0.47	0.46	0.02
Yangling, China (CHI)	9.88	4.25	3	2.47	0.65	0.56	0.14
Shimla, India (IND)	19.75	3.38	0	2.36	0.59	0.50	0.16
Bangkok, Thailand (THA)	8.75	3.75	1	2.21	0.63	0.37	0.41
Mean average	17.46	3.70	1.27	2.21	0.58	0.44	0.23

coefficients (*F*) varying from 0.06 (population BR3) to 0.46 (population THA). Overall mean was 0.23. A total of 19 private alleles were identified. These private alleles were found in ten populations: BR1, COL, MEX, and THA (a single private allele per population); BR4, BR6, and USA (two private alleles per population); and BR7, CHI, and MOZ (three private alleles per population) (table 3). Results of the AMOVA revealed that differences within populations accounted for 81.5% of the total variance. The genetic differentiation was moderate among all populations ($F_{ST} = 0.115$, $G_{ST} = 0.110$, $R_{ST} = 0.105$).

The analysis carried out on structure revealed that the 309 specimens of the maize weevil contained ancestry in three Bayesian groups (best $K = 3$). Overall, specimens from any given population displayed varying proportions of membership coefficients in the three Bayesian groups – depicted in gray, orange, and blue; respectively (fig. 4). Most of the 15 populations contained specimens that displayed proportion of membership in at least two Bayesian groups: more noticeable the joint presence of the gray and orange Bayesian groups, which were widespread in the Americas and Asia. There were populations in which most of the specimens exhibited a high proportion of membership in a single Bayesian group. For example, four populations from the Americas (EUA, MEX, PAN, and BR7) together with two populations of Asia (CHI and THA) showed the highest proportion of membership assigned to the gray Bayesian group, which ranged from 83% (EUA) to 40% (BR7). Six populations from Brazil (BR1 to BR6) and the population from India (IND) showed the highest proportion of assignment in the orange group, ranging from 76% (BR4) to 52% (IND). The presence of the third Bayesian group (depicted in blue) was most obvious in populations COL (90%) and MOZ (81%), which were each collected from sites located very far apart: Colombia and Mozambique. These results provided evidence for the lack of spatial genetic structure at the regional, continental, and intercontinental levels and suggested high gene flow amongst maize weevil populations.

Discussion

The maize weevil and the rice weevil are each a distinct species

Our molecular evidence suggests that speciation is complete; therefore, the maize weevil should not be considered as a derived strain of its 'sibling species' – the rice weevil. Although they share a great deal of morphological similarities (Kuschel, 1961; Halstead, 1963), the maize weevil and the rice weevil are reproductively isolated and therefore distinct species (Hidayat *et al.*, 1996). Molecular evidence for complete speciation comes from the fact that there was no haplotypes shared between species; moreover, the networks displayed discontinuous distributions of haplotypes, with large gaps between groups within each network. Each species exhibited its own mitogroup (for the mitochondrial genome) and ribogroup (for the nuclear genome). The large mutational distances – 167 steps between mitogroups; 39 steps between ribogroups – indicate that gene flow between species has ceased completely. Since the time of their split from the tMRCA, the two species evolved without further genetic admixture. Over time, non-shared mutations accumulated within each mitogroups (or ribogroup) and gave rise to the large gaps uncovered on the networks. The large pairwise differences in the COI genes allowed for the use of molecular tools to discriminate between weevil species (Hidayat *et al.*, 1996; Corrêa *et al.*, 2013).

Ancient origin of the maize weevil

Molecular data provide support for an ancient origin of the maize weevil – about 8.7 My ago, with the 95% HPD lower bound at 4.0 My ago, which is significantly older than the establishment of agriculture between 10,000 and 6000 yBP. Our estimated time of divergence is very coarse and should be taken with caution, given that it was obtained with sequence data from a single gene set and used a mean substitution rate (0.0177 substitutions per site per My) estimated for the COI gene of beetles (Papadopoulou *et al.*, 2010). Estimating precisely when the maize weevil and the rice weevil split from the tMRCA is not a simple task. Although with caveats, our estimated time of divergence is valuable because it shed light on the temporal placement of the origin of the maize weevil relative to the onset of agriculture. This is compelling evidence that both the maize weevil and the rice weevil emerged as independent species prior to the onset of agriculture.

Additionally, in order to constrain the origin of the maize weevil within the time frame maize cultivation left Mesoamerica (about 400 years ago), the divergence dating

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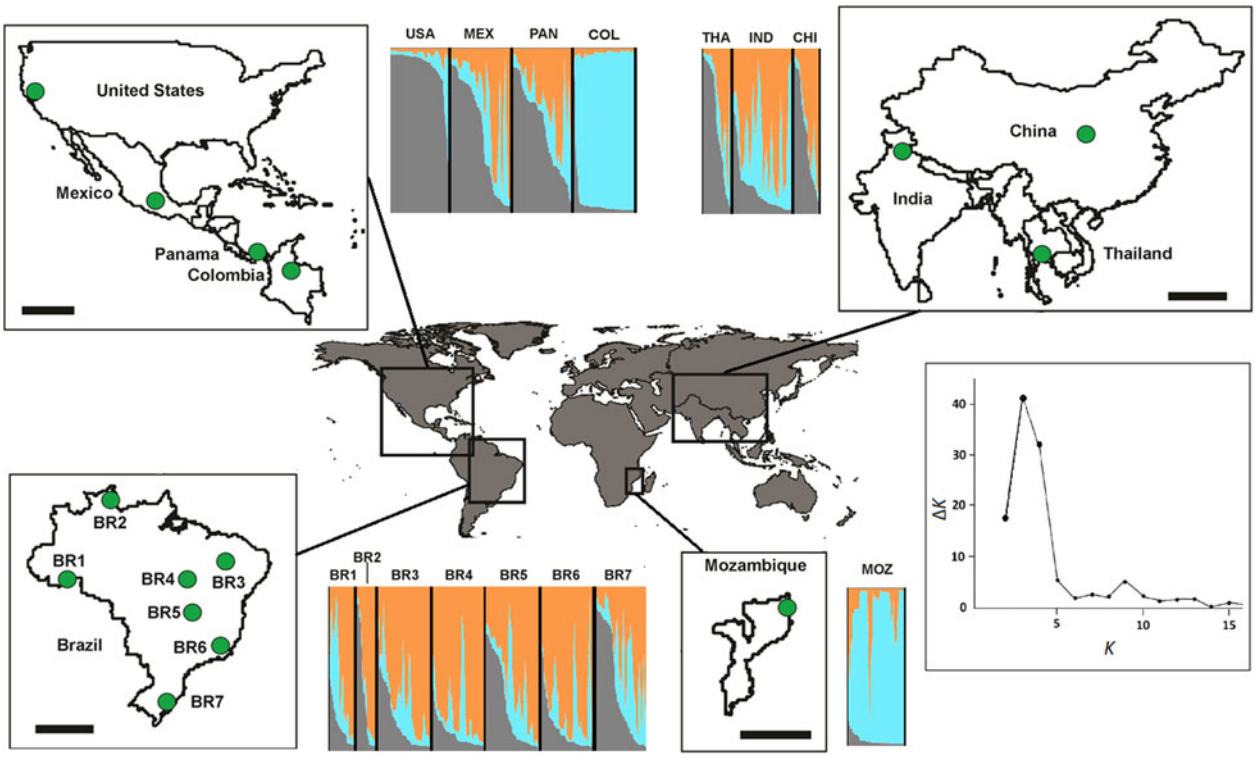

Fig. 4. Clustering analyses based on eight microsatellite loci for 309 specimens of maize weevil (*Sitophilus zeamais*) and geographic distribution of the three Bayesian groups (coded gray, orange, or blue) across 15 study populations. Along the *x*-axis of each plot, each vertical bar represents a weevil specimen; along the *y*-axis, membership coefficient of a specimen for a Bayesian group represents the fraction of its genome that has ancestry in that Bayesian group. In the insert, the best *K* (*K* = 3) calculated according to the Δ*K* method (Evanno *et al.*, 2005).

analysis would require the mean substitution rate for the COI gene of *Sitophilus* to run 20,000 faster (354 substitutions per site per My) than the fastest known rate for the COI gene of beetles (0.0177 substitutions per site per My) (Papadopoulou *et al.*, 2010). This is compelling evidence that the maize weevil emerged prior to the human-mediated dissemination of maize as part of the Columbian Exchange (Crosby, 2003).

Our results do not allow us to deduce where the ancestral ranges of the maize weevil resided. A body of evidence suggests that both Southeast Asia and the Indian subcontinent are likely regions where the maize weevil may have emerged. These regions provide home for the remaining, non-pest species of *Sitophilus* (Plarre, 2010). Archeological findings attest the early association of *Sitophilus* with human activities in Southeast Asia. Deposits of ca. 3000 yBP in China contained *Sitophilus* specimens, which very likely are rice weevils (Chu & Wang, 1975; Buckland, 1981). Pottery dated from ca. 10,500 yBP in Japan showed impressions of *Sitophilus*, which the authors claimed as belonging to maize weevils (Obata *et al.*, 2011). We hypothesize that the early cultivation and storage systems of rice and other cereal on Asia may have led to a habitat shift in the grain, from the prior acorn-feeding on forest environments to the later stored grains from early agricultural settlements.

Recent association between maize and maize weevil

Most of today's leading cereals were domesticated on the Old World; maize is an exception. Maize has been domesticated in the Mexican highlands about 9000 yBP (Piperno *et al.*, 2009; Matsuoka *et al.*, 2002). During the next few millennia, its cultivation quickly spread northwards into North America and southwards into the Andes and the Atlantic coast of South America, including Brazil (Iriarte *et al.*, 2004; Pohl *et al.*, 2007). Currently, maize has the broadest cultivation range of all cereals (Van Heerwaarden *et al.*, 2011; Mir *et al.*, 2013). The timing of which maize weevil became associated with maize is intriguing, given that the origin of the pest insect took place likely on Asia, whereas maize originated on Mesoamerica.

Archeological records that could attest the presence of *Sitophilus* on Mesoamerica prior to the Columbian Exchange are silent. Without human intervention, it is highly unlikely that the maize weevil could leave its ancestral ranges on Asia, expand its geographical range to Mesoamerica through a transoceanic journey, and finally find suitable habitats at early maize cultivation systems. Had the maize weevil left Asia and arrived on Mesoamerica prior to – or immediately after – maize domestication, our microsatellite analyses would had uncovered the signatures of this geographic range expansion on the population structure of the maize weevil. In contrast, we found no molecular evidence to support the Americas as a source of genetic variation for other geographic regions; there was no phylogeographic signals preserved on a region-dependent manner. The fact that genetic variation of the American populations reached values similar to those found elsewhere is consistent with the Americas as part of

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recent colonization events associated with high gene flow (see next section for details). Thus, we hypothesize that the maize weevil became associated with maize only very recently; this association took place after maize became widespread as a global crop. It is plausible that the relatively large grain size of maize and the warmer conditions in which maize is cultivated favored the maize–maize weevil association (Van Heerwaarden *et al.*, 2011; Corrêa *et al.*, 2013; Mir *et al.*, 2013).

Weak spatial structure at diverse geographic scales

Microsatellite marker analyses yielded concordant results with DNA sequence data. However, microsatellite markers analyses provided additional details about the distribution of genetic diversity of the maize weevil across cultivation areas at the regional, continental, and intercontinental scales that were not noticeable from DNA sequence data alone.

Sampling maize weevil across Brazil for microsatellite analyses – 142 specimens from seven populations that cover most of the maize cultivation area in the country – allowed us to investigate extant levels of gene flow among populations at the regional level. The lack of spatial genetic structure in Brazil is consistent with a pest species that: (a) has been introduced recently – more likely during the last few hundred years; (b) is capable of dispersing to short distances owing to its flying capabilities (Throne & Cline, 1989; Rees, 1996); (c) is capable of reaching new, distant territories owing to human-mediated transportation of infested grains (Longstaff, 1981; Throne & Cline, 1989; Plarre, 2010). High levels of gene flow and low genetic differentiation among populations constitute a pattern that very likely is not restricted to the maize weevil populations from Brazil; his pattern is probably widespread at a regional scale across other maize cultivation areas around the world (Semeao *et al.*, 2012; Coelho-Bortolo *et al.*, 2016; Thagaraj *et al.*, 2016).

To explore the spatial genetic structure of the maize weevil further, we analyzed data from additional populations from the Americas (USA, MEX, PAN, and COL), Africa (MOZ), and Asia (IND, CHI, and THA). The lack of spatial genetic structure that has been detected at the regional level (Brazilian populations) was also found at the continental and intercontinental levels, with two Bayesian groups dispersed worldwide. The restricted distribution of one of the three Bayesian groups (coded blue in fig. 4) indicated a recent gene flow between Colombia and Mozambique. Direction of this gene flow could not be determined using our current dataset, but the Colombia–Mozambique pattern of gene flow is consistent with the major role that Colombia played in the Portuguese slave trade possibly leading to maize introduction from Colombia to Africa, including Mozambique (Madeira Santos & Ferraz Torrão, 1998; Mir *et al.*, 2013). Altogether, these findings suggest that the maize weevil underwent recent range expansions at a global scale, most likely mediated through human-related activities.

The lack of spatial genetic structure at varying geographic levels has implications for pest management. Physiological and behavioral differences among populations of the maize weevil have been reported and such differences incur in likely differences in dispersion and damage potential to the host grains (Morales *et al.*, 2013; Carvalho *et al.*, 2014; Malia *et al.*, 2016). However, such differences seem more prevalent within populations rather than between populations, suggesting a high multidirectional gene flow, which is consistent with the findings we reported herein and with recent studies using sets of integrated behavioral tendencies in populations of the maize weevil (Morales *et al.*, 2013; Malia *et al.*, 2016).

Phytosanitary concerns for the maize weevil

Grain weevils are very damaging to stored cereals. While the maize weevil is particularly important as a pest of stored maize on both Neotropical America and Africa, the rice weevil is important for stored maize in temperate climate regions such as North America, Europe, and Asia (Longstaff, 1981; Rees, 1996; Plarre, 2010; Corrêa *et al.*, 2013). The maize weevil exhibits a range of trait variations to support its status as insect pest, including flight proficiency, walking ability, body mass, grain consumption, and genes for insecticide resistance (Grenier *et al.*, 1994; Daglish, 2004; Guedes *et al.*, 2006, 2010; Corrêa *et al.*, 2011). The unintended introduction of novel genotypes may increase the fitness of local populations of the maize weevil because the newly introduced specimens may carry a vast array of favorable alleles. Upon mating, reshuffling of the newly acquired genes together with the genes of the local specimens may give rise to novel genomic combinations, which otherwise would not take place on either local or regional populations of the maize weevil. Thus, phytosanitary agencies should pay attention to prevent, at the maximum extent possible, the introduction of novel genetic variation, especially genetic variation with origin from populations that might have experienced high selection pressure toward adaptive traits.

Enhanced dispersal ability and insecticide resistance are some of the traits that may potentially aggravate grain losses when specimens that harbor them are re-introduced into occupied territories or introduced into newly occupied areas. Insecticide use and insecticide resistance of maize weevil for instance are frequent problems in Neotropical America (Champ & Dyte, 1977; Subramanyam & Hagstrum, 1996; Ribeiro *et al.*, 2003; Pimentel *et al.*, 2009; Haddi *et al.*, 2015). Unintended human-mediated transport may disperse adaptive traits, eventually leading to the establishment of insecticide resistant genotypes of the weevils elsewhere. Therefore, phytosanitary measures to prevent further transfer of both the maize weevil and the rice weevil, particularly from warm maize producing areas, are worthwhile and should be implemented.

In conclusion, we uncovered evidence for an ancient origin of both the maize weevil and its morphologically indistinguishable congener, the rice weevil; both species likely emerged prior to the beginning of the agriculture on Asia. The association between the maize weevil with maize, its preferred host, was established recently, after maize cultivation became widespread. Lack of spatial structure characterized the extant populations of the maize weevil, which is suggestive of high gene flow.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0007485316000687.

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References

Akaike, H. (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19**, 716–723.

Bandelt, H.J., Forster, P. & Rohl, A. (1999) Median–joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**, 37–48.

Barat, A., Bravo, S.P., Chandra, S., Corrêa, A.S., Giombini, M.I., Guedes, R.N., Huailei, M., Lal, K.K., Liang, L., Matura, R., Mohindra, V., Oliveira, L.O., Patangia, R., Qiyong, L., Sah, R.S., Singh, A., Singh, B.K., Singh, R.K., Tosto, D.S., Tripathi, R.K. & Vinson, C.C. (2012) Permanent genetic resources added to molecular ecology resources database 1 June 2012–31 July 2012. *Molecular Ecology Resources* **12**, 1196–1197.

Brown, T.A., Jones, M.K., Powell, W. & Allaby, R.G. (2009) The complex origins of domesticated crops in the Fertile Crescent. *Trends in Ecology & Evolution* **24**, 103–109.

Buckland, P.C. (1981) The early dispersal of insect pests of stored products as indicated by archeological records. *Journal of Stored Products Research* **17**, 1–12.

Carvalho, G.A., Vieira, J.L., Haro, M.M., Corrêa, A.S., Ribon, A. O.B., Oliveira, L.O. & Guedes, R.N.C. (2014) Pleiotropic impacto f endosymbiont load and co-occurrence in the maize weevil *Sitophilus zeamais*. *PLoS ONE* **9**(10), e111396.

Champ, B.R. & Dyte, C.E. (1977) FAO global survey of pesticide susceptibility of stored grain pests. *FAO Plant Protection Bulletin* **25**, 49–67.

Chu, H.F. & Wang, L.Y. (1975) Insect carcasses unearthed from Chinese antique tombs. *Acta Entomologica Sinica* **18**, 333–337.

Clark, T.L., Meinke, L.J. & Foster, J.E. (2001) Molecular phylogeny of *Diabrotica* beetles (Coleoptera: Chrysomelidae) inferred from analysis of combined mitochondrial and nuclear DNA sequences. *Insect Molecular Biology* **10**, 303–314.

Coelho-Bortolo, T., Mangolin, C.A. & Lapenta, A.S. (2016) Genetic Variability in the natural populations of *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), detected by RAPD markers and the esterase isozymes. *Bulletin of Entomological Research* **106**, 47–53.

Corrêa, A.S., Pereira, E.J.G., Cordeiro, E.M.G., Braga, L.S. & Guedes, R.N.C. (2011) Insecticide resistance, mixture potentiation and fitness in populations of the maize weevil (*Sitophilus zeamais*). *Crop Protection* **30**, 1655–1666.

Corrêa, A.S., Oliveira, L.O., Braga, L.S. & Guedes, R.N.C. (2013) Distribution of the related weevil species *Sitophilus oryzae* and *S. zeamais* in Brazil. *Insect Science* **20**, 763–770.

Corrêa, A.S., Tomé, H.V.V., Braga, L.S., Martins, G.F., Oliveira, L.O. & Guedes, R.N.C. (2014) Are mitochondrial lineages, mitochondrial lysis and respiration rate associated with phosphine susceptibility in the maize weevil *Sitophilus zeamais*? *Annals of Applied Biology* **165**, 137–146.

Cotton, R.T. (1920) Tamarind Pod Borer, *Sitophilus linearis* (Hbst.). *Journal of Agricultural Research* **20**, 439–446.

Crandall, K.A. & Templeton, A.R. (1993) Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* **134**, 959–969.

Crosby, A.W. (2003) *The Columbian Exchange: Biological and Cultural Consequences of 1492*. Westport, USA, Praeger.

Daglish, G.J. (2004) Effect of exposure period on degree of dominance of phosphine resistance in adults of *Rhyzopertha dominica* (Coleoptera: Bostrychidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae). *Pest Management Science* **60**, 822–826.

Drummond, A.J., Suchard, M.A., Xie, D., & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**, 1969–1973.

Earl, D.A. & vonHoldt, B.M. (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4**, 359–361.

Evanno, G., Regnaut, S. & Goudet, J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**, 2611–2620.

Excoffier, L., Smouse, P.E. & Quattro, J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479–491.

Excoffier, L., Laval, G. & Schneider, S. (2006) *ARLEQUIN Version 3. 1: an Integrated Software Package for Populations Genetics Data Analysis*. Bern, Switzerland, University of Bern. http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.132.1270.

Fuller, D.Q., Harvey, E. & Qin, L. (2007) Presumed domestication? Evidence for wild rice cultivation and domestication in the fifth millennium BC of the Lower Yangtze region. *Antiquity* **81**, 316–331.

Goudet, J. (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity* **86**, 485–486.

Goudet, J. (2002) *FSTAT: a Computer Program to Calculate F Statistics. Version 2.9.3.2*. http://www2.unil.ch/popgen/softwares/fstat.htm.

Grenier, A.M., Nardon, C. & Nardon, P. (1994) The role of symbiotes in flight activity of *Sitophilus* weevils. *Entomologia Experimentalis et Applicata* **70**, 201–208.

Guedes, N.M.P., Tolledo, J., Corrêa, A.S. & Guedes, R.N.C. (2010) Insecticide-induced hormesis in an insecticide-resistant strain of the maize weevil, *Sitophilus zeamais*. *Journal of Applied Entomology* **134**, 142–148.

Guedes, R.N.C., Oliveira, E.E., Guedes, N.M.P., Ribeiro, B. & Serrão, J.E. (2006) Cost and mitigation of insecticide resistance in the maize weevil, *Sitophilus zeamais*. *Physiological Entomology* **31**, 30–38.

Haddi, K., Mendonça, L.P., Santos, M.F., Guedes, R.N.C. & Oliveira, E.E. (2015) Metabolic and behavioral mechanisms of indoxacarb resistance in *Sitophilus zeamais* (Coleoptera: Curculionidae). *Journal of Economic Entomology* **108**, 362–369.

Haines, C.P. (1981) *Insects and Arachnids from Stored Products: a Report on Specimens Received by the Tropical Stored Products Center*. London, TDRI, pp. 1973–1977.

Halstead, D.G.H. (1963) External sex differences in stored-product Coleoptera. *Bulletin of Entomological Research* **54**, 118–134.

Hidayat, P., Phillips, T.W., & Ffrench-Constant, R.H. (1996) Molecular and morphological characters discriminate *Sitophilus oryzae* and *S. zeamais* (Coleoptera: Curculionidae)

and confirm reproductive isolation. *Annals of the Entomological Society of America* **89**, 645–652.

Iriarte, J., Holst, I., Marozzi, O., Listopad, C., Alonso, E., Rinderknecht, A. & Montaña, J. (2004) Evidence for cultivar adoption and emerging complexity during the mid-Holocene in the La Plata basin. *Nature* **432**, 614–617.

Jakobsson, M. & Rosenberg, N.A. (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure, version 1.1. *Bioinformatics* **23**, 1801–1806.

Kealhofer, L. & Piperno, D.R. (1994) Early agriculture in southeast Asia: phytolith evidence from Bang Pakong Valley, Thailand. *Antiquity* **68**, 564–572.

Kiritani, K. (1956) On the local distribution of two allied species of the rice weevils *Calandra oryzae* and *C. sasaki*. *Japanese Journal of Applied Zoology* **21**, 74–77.

Kuschel, G. (1961) On problems of synonymy in the *Sitophilus oryzae* complex (30th contribution, Col. Curculionidae). *Annals and Magazine of Natural History: Ser. 13* **4**, 241–244.

Levinson, H. & Levinson, A. (1994) Origin of grain storage and insect species consuming desiccated food. *Anz Schädl kd Pflanzenschutz Umweltschutz* **67**, 47–59.

Lev-Yadun, S., Gopher, A. & Abbo, S. (2000) The cradle of agriculture. *Science* **288**, 1602.

Lewis, P.O. & Zaykin, D. (2001) *Genetic Data Analysis: Computer Program for the Analysis of Allelic Data*. 1.0 ed. http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php (accessed December 2013).

Librado, P.O. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451–1452.

Longstaff, B.C. (1981) Biology of the grain pest species of the genus *Sitophilus* (Coleotpera: Curculionidae): a critical review. *Protection Ecology* **2**, 83–130.

Lopez, J.V., Yuhki, N., Masuda, R., Modi, W. & O'Brien, S.J. (1994) Numt, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat. *Journal of Molecular Evolution* **39**, 174–190.

Madeira Santos, M.E. & Ferraz Torrão, M.M. (1998) Entre lÁmérrique et l'Afrique, les îles du Cap-Vert et São Tomé: les cheminements des milhos (mil, sorgho et maïs). pp. 69–83 *in* Chastanet, M. (*Ed.*) *Plantes et Paisages d'Afrique. Une Historie à Explorer*. Paris, Karthala-CRA.

Malia, H.A.E., Rosi-Denadai, C.A., Cardoso, D.G. & Guedes, R. N.C. (2016) Dust to weevils, weevils to dust: maize weevil personality and susceptibility to diatomaceous earth. *Journal of Pest Science* **89**, 469–478.

Matsuoka, Y., Vigouroux, Y., Goodman, M.M., Sanchez, J., Buckler, E. & Doebley, J. (2002) A single domestication for maize shown by multilocus microsatellite genotyping. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 6080–6084.

Mir, C., Zerjal, T., Combes, V., Dumas, F., Madur, D., Bedoya, C., Dreisigacker, S., Franco, J., Grudloyma, P., Hao, P.X., Hearne, S., Jampatong, C., Laloë, D., Muthamia, Z., Nguyen, T., Presanna, B.M., Taba, S., Xie, C.X., Yunus, M., Zhang, S., Warburton, M.L. & Hearne, S. (2013) Out of America: tracing the genetic footprints of the global diffusion of maize. *Theoretical and Applied Genetics* **126**, 2671–2682.

Morales, J.A., Cardoso, D.G., Della Lucia, T.M.C. & Guedes, R. N.C. (2013) Weevil x insecticide: does "personality" matter? *PLoS ONE* **8**, e67283.

Nei, M. (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America* **70**, 3321–3323.

Nylander, J.A.A. (2004) *MrModeltest v2. Program Distributed by the Author*. Uppsala, Sweden, Evolutionary Biology Centre, Uppsala University.

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**, e14785.

Oliveira, M.R.C., Corrêa, A.S., de Souza, G.A., Guedes, R.N.C. & de Oliveira, L.O. (2013) Mesoamerican origin and pre- and post-Columbian expansions of the ranges of *Acanthoscelides obtectus* Say, a cosmopolitan insect pest of the common bean. *PLoS ONE* **8**, e70039.

Panagiotakopulu, E. (2001) New records for ancient pests: archaeoentomology in Egypt. *Journal of Archaeological Science* **28**, 1235–1246.

Papadopoulou, A., Anastasiou, I. & Vogler, A.P. (2010) Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. *Molecular Biology and Evolution* **27**, 1659–1672.

Peng, W.K., Lin, H.C., Chen, C.N. & Wang, C.H. (2003) DNA identification of two laboratory colonies of the weevils, *Sitophilus oryzae* (L.) and *S. zeamais* Motschulsky (Coleoptera: Curculionidae) in Taiwan. *Journal of Stored Products Research* **39**, 225–235.

Pimentel, M.A.G., Faroni, L.D.A., Guedes, R.N.C., Sousa, A.H. & Tótola, M.R. (2009) Phosphine resistance in Brazilian populations of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *Journal of Stored Products Research* **45**, 71–74.

Piperno, D.R., Ranere, A.J., Holst, I., Iriarte, J. & Dickau, R. (2009) Starch grain and phytolith evidence for early ninth millennium BP maize from the Central Balsas River Valley, Mexico. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 5019–5024.

Plarre, R. (2010) An attempt to reconstruct the natural and cultural history of the granary weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae). *European Journal of Entomology* **107**, 1–11.

Pohl, M.E., Piperno, D.R., Pope, K.O. & Jones, J.G. (2007) Microfossil evidence for pre-Columbian maize dispersals in the neotropics from San Andres, Tabasco, Mexico. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 6870–6875.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Reference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

Rees, D.P. (1996) Coleoptera. pp. 1–39 *in* Subramanyam, B. & Hagsrum, D.W. (*Eds*) *Integrated Management of Insects in Stored Products*. New York, Marcel Dekker.

Ribeiro, B.M., Guedes, R.N.C., Oliveira, E.E. & Santos, J.P. (2003) Insecticide resistance and synergism in Brazilian populations of *Sitophilus zeamais* (Coleoptera: Curculionidae). *Journal of Stored Products Research* **39**, 21–31.

Richards, O.W. (1944) The two strains of the rice weevil Calandra oryzae (L.) (Coleoptera Curculionidae). *Transactions of the Royal Entomological Society of London* **94**, 187–200.

Semeao, A.A., Campbell, J.F., Beeman, R.W., Lorenzen, M.D., Whitworth, R.J. & Sloderbeck, P.E. (2012) Genetic structure of *Tribolium castaneum* (Coleoptera: Tenebrionidae) populations in mills. *Environmental Entomological* **41**, 188–199.

Slatkin, M. (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139**, 457–462.

Solomon, M.E. (1965) Archaeological records of storage pests: *Sitophilus granarius* (L.) (Coleoptera, Curculionidae) from an Egyptian pyramid tomb. *Journal of Stored Products Research* **1**, 105–107.

Subramanyam, B.H. & Hagstrum, D.W. (1996) *Integrated Management of Insects in Stored Products*. Boston, MA, Marcel Dekker.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**, 2731–2739.

Thagaraj, S.R., McCulloch, G.A., Subbarayalu, M., Subramaniam, C. & Walter, G.H. (2016) Development of microsatellite markers and a preliminary assessment of population structuring in the rice weevil, *Sitophilus oryzae* (L.). *Journal of Stored Products Research* **66**, 12–17.

Throne, J.E. & Cline, L.D. (1989) Seasonal flight activity of the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), and the rice weevil, *S. oryzae* (L.), in south Caroline. *Journal of Agricultural Entomology* **6**, 183–192.

Throne, J.E. & Cline, L.D. (1991) Seasonal abundance of maize and rice weevils (Coleoptera: Curculionidae) in South Caroline. *Journal of Agricultural Entomology* **8**, 93–100.

van Heerwaarden, J., Doebley, J., Briggs, W.H., Glaubitz, J.C., Goodman, M.M., Gonzalez, J.D.J.S. & Ross-Ibarra, J. (2011) Genetic signals of origin, spread, and introgression in a large sample of maize landraces. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 1088–1092.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. & Shipley, P. (2004) MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535–538.

Weir, B.S. & Cockerham, C.C. (1984) Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. pp. 315–322 *in* Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (*Eds*) *PCR Protocol: a Guide to Methods and Applications*. New York, Academic Press.