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Ancient and recent evolutionary history of the bruchid beetle, *Acanthoscelides obtectus* Say, a cosmopolitan pest of beans

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Abstract

Acanthoscelides obtectus* Say is a bruchid species of Neotropical origin, and is specialized on beans of the *Phaseolus vulgaris* L. group. Since the domestication and diffusion of beans, *A. obtectus* has become cosmopolitan through human-mediated migrations and is now a major pest in bean granaries. Using phylogeographic methods applied to mitochondrial DNA (mtDNA) and nuclear microsatellite molecular markers, we show that the origin of this species is probably further south than Mesoamerica, as commonly thought. Our results also indicate that *A. obtectus* and its Mesoamerican sister species *Acanthoscelides obvelatus*, two morphologically close species differing principally in voltinism, speciated in allopatry: *A. obtectus* (multivoltine) arising in Andean America and *A. obvelatus* (univoltine) in Mesoamerica. In contrast to Mesoamerica where beans fruit once yearly, wild beans in Andean America fruit year-round, especially in regions showing little or no seasonality. In such habitats where resources are continuously present, multivoltinism is adaptive. According to existing hypotheses, multivoltinism in *A. obtectus* is a new adaptation that evolved after bean domestication. Our data suggest the alternative hypothesis that multivoltinism is an older trait, adapted to exploit the year-round fruiting of wild beans in relatively aseasonal habitats, and allowed *A. obtectus* to become a pest in bean granaries. This trait also permitted this species to disperse through human-mediated migrations associated with diffusion of domesticated beans. We also show that diversity of Old World *A. obtectus* populations can be quite well explained by a single colonization event about 500 BP. Human-mediated migrations appear not to be rare, as our results indicate a second more recent migration event from Andean America to Mexico.

Keywords: *Acanthoscelides obtectus*, Coleoptera, human-mediated migrations, pest species, *Phaseolus*, phylogeography

Introduction

Bruchid beetles (Bruchidae, Coleoptera) are seed-eating insects, most species of which feed on legumes. Among the more than 1500 bruchid species described assigned to 58 different genera (Borowiec 1987), a substantial percentage are specialized on economically important plant species. Most of these bruchid species now have worldwide distributions, as a result of movement in stocks of seeds, which have undergone very long distance migrations through human exchanges at the intra- and intercontinental levels.

Acanthoscelides obtectus Say is a bruchid species of Neotropical origin, specialized on beans of the *Phaseolus vulgaris* L. group (see Delgado-Salinas *et al.* 1999). Understanding the native and introduced range of this species and the phylogeographic relationships among populations is of scientific interest in itself, particularly for the study of the consequences of past and present human actions — involving for example migration patterns — on

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the current structure and biology of populations. The role of human-mediated migrations is of particular importance in understanding the biogeographical history of *A. obtectus*, as its dispersal capacity has been artificially increased since the domestication of *Phaseolus* beans about 7000 BP (Kaplan 1971).

Understanding phylogeographic patterns in the species is also of particular agronomic concern, because knowledge of the native range of a species may give new data on the climatic conditions in which the species originally evolved, and can also otherwise guide the search for agents of biological control. *A. obtectus* is now cosmopolitan, and has become a frequent pest in bean granaries. In developing countries, farmers who produce *Phaseolus* beans experience significant losses because of the high reproductive rate of *A. obtectus*, and its ability to reproduce in a broad range of ecological conditions (Labeyrie 1981), including tolerance to large amounts of several kinds of pesticides (N. Alvarez, unpublished). In the last 30 years, *A. obtectus* has been reported on new host plant species, among them several domesticated legumes such as *Cajanus indicus*, *Vigna unguiculata*, *Pisum sativum* and *Vicia faba* (Johnson 1983, 1990). This modern expansion of the geographical and host range of *A. obtectus* thus threatens production of other crops, and there is an urgent need to control bruchid populations by other than chemical means.

Most authors situate the origin of *A. obtectus* in Mesoamerica (e.g. Labeyrie 1990). Based on this assumption, entomologists and agronomists have started scientific programs in Mexico and Central America (south to northern Colombia) to find candidate parasitoid species for biological control (e.g. Schmale *et al*. 2001). However, results have so far been disappointing, and to our knowledge Mesoamerican species of parasitoids have never been able to reduce densities of *A. obtectus* in seed stocks (Schmale *et al*. 2002). The challenge to find an appropriate biological control agent originating in the Neotropical region, and initially adapted to *A. obtectus*, is thus of current interest.

The assumption that *A. obtectus* originates from Mexico is not based on strong arguments. The contemporary range of *A. obtectus* in the New World extends from Chile to the United States (Johnson 1983, 1990), and the original range of the species may have been further south, rather than in Mesoamerica as currently believed. One of the objectives of this study was thus to examine the phylogeography of *A. obtectus* in the Neotropics, to gain insights into its area of origin.

Acanthoscelides obvelatus Bridwell is the sister species of *A. obtectus*, and also feeds on beans of the *P. vulgaris* group. These two species are very similar morphologically. A few anatomical and morphological characters make their differentiation possible (Leroi *et al*. 1990; Alvarez *et al*. 2004a), but confusion between the two species can still easily arise, especially when they occur in sympatry. The most important biological difference between the two species is in their voltinism. Whereas *A. obtectus* can continue to reproduce as long as resources are available, *A. obvelatus* only produces a single generation per year, in synchrony with the fruiting of beans. This trait of *A. obvelatus* precludes long-distance human-mediated migrations like those undertaken by *A. obtectus*, and *A. obvelatus* is today still restricted to Central America and extreme northern South America [the species has been recorded only from Mexico, Guatemala and northern Colombia (Johnson 1983)]. Furthermore, *A. obvelatus* has never been recorded from other species than beans of the *P. vulgaris* group.

The important difference in voltinism has led several authors to suppose that the two species speciated sympatrically (e.g. Pichard *et al*. 1991). According to this hypothesis, *A. obvelatus* has remained adapted to the phenology of wild bean populations, whereas *A. obtectus* has evolved multivoltinism as an adaptation to reproduction in granaries. This hypothesis implies a very recent speciation, since bean domestication occurred around 7000 BP (Kaplan 1971; Kaplan & Lynch 1999). In a recent study, Alvarez *et al*. (2004a) provided strong arguments for a much more ancient separation between the two species. The extent of divergence in *COI* sequences of *A. obtectus* and *A. obvelatus* (16.7%) indicates that the separation between these two species is at least 20 Ma. In this study, we will provide new molecular data that give insights on their origin. This information suggests a new scenario, which points to a decisive role played by human-mediated migrations. An implication of this scenario is that entomologists and agronomists should extend the search for candidate species for use in biological control to South America.

Materials and methods

Specimens of *Acanthoscelides obtectus* were collected between December 2001 and June 2003 from sites in Mexico, Peru, France, Switzerland, Spain, and Cameroon (see Table 1).

Mitochondrial DNA

To examine the phylogeographic pattern of the species, we sequenced three mitochondrial genes [*12s rRNA* (379 bp), *16s rRNA* (465 bp) and *COI* (736 bp)], from 14 sites (one individual per site, with the exception of the Peruvian site with 10 individuals): Mexico (9 sites), Peru (1 site), Cameroon (1 site), Switzerland (1 site), France (1 site), and Spain (1 site). Old World specimens were all collected in cultivated bean fields, whereas specimens from the New World originated either in cultivated bean fields (Peru; Mexico COE, SFP, XOT, YOH), or in wild bean populations (Mexico SAG, SCA, SJS, STO, TLA). Sampled sites in Mexico are situated in the Mesoamerican altiplano, which is considered to be a centre of diversity for

Table 1 List of sampled sites

Abbrev.	Site name	Kind of bean population	Longitude	Latitude	Altitude (m)
COE	Coeneo, Mexico	d	101°34′59.3″W	19°49′13.9″N	2100
MAL	Malinalco, Mexico	w	99°30′08.9″W	18°57′13.2″N	1935
SAG	San Andres de los Gabeles, Mexico	w	99°57′01.5″W	19°02′19.5″N	2280
SCA	San Calletano, Mexico	w	100°05′51.4″W	19°22′09.1″N	2611
SFP	San Francisco Periban, Mexico	d	102°24′28.4″W	19°32′32.4″N	1800
SIC	San Isidro Coeneo, Mexico	d	101°34′23.9″W	19°50′56.6″N	2040
SJS	San Jose de los Laureles, Mexico	w	99°00′05.0″W	18°58′49.7″N	1855
STM	Santa Maria, Mexico	d	99°33′42.7″W	18°49′03.6″N	2000
STO	Santo Tomas, Mexico	w	100°16′30.2″W	19°11′20.0″N	1390
TEJ	Tejupilco, Mexico	w	100°09′00.1″W	18°55′51.2″N	1400
TEP	Tepoztlan, Mexico	w	99°07′15.7″W	18°59′36.3″N	1931
TLA	Tlalpan, Mexico	w	99°12′04.3″W	19°17′50.3″N	2403
XOT	Xochitlan, Mexico	d	97°39′01′2″W	19°57′59.9″N	1450
YOH	Yohualichan, Mexico	d	97°30′55.9″W	20°00′56.0″N	1400
MAM	Mamorimamba, Peru	d	78°48′33.6″W	6°30′42.2″S	1900
YAH	Cameroon	d	Yaoundé, Province du Centre		
BEL	France	d	Bellevue, Poitou		
CHA	France	d	Chail, Poitou		
NEU	Switzerland	d	Chambrelien, Canton de Neuchâtel		
PRA	Spain	d	Prado, Provincia de León		

Kind of bean population: d, domestic; w, wild; altitude (m), meter above sea level.

beans (e.g. Delgado-Salinas *et al*. 1988). The Peruvian site was situated in Mamorimamba, in the Departamento de Cajamarca. Origins of the Old World sampled sites were as follows: Spain, Provincia de Leon; France, Région du Poitou; Switzerland, Canton de Neuchâtel; Cameroon, Province du Centre. *Acanthoscelides argillaceus* Sharp and *Acanthoscelides obvelatus* were used as outgroup species. To compare the genetic diversity of *A. obtectus* in Mexico to that of its sister species *A. obvelatus*, we sequenced the mitochondrial *COI* gene from 16 *A. obtectus* and 18 *A. obvelatus* specimens (originating from 8 and 11 sites, respectively). Primer sequences were defined according to Simon *et al*. (1994): *12Sbi* (AAGAGCGACGGGCGATGTGT) and *12Sai* (AAACTAGGATTAGATACCCTATTAT) for *12s rRNA*; modified *16Sbr* (CCGGTTTGAACTCAGATCATGT) and modified *16Sar* (CGCCTGTTTAACAAAAACAT) for *16s rRNA*; *C1-J-2183* (CAACATTTATTTTGATTTTTTGG) and modified *TL2-N-3014* (TCCATTGCACTAATCTGCCATATTA) for *COI*. Total genomic DNA was extracted using DNeasy Kit (QIAGEN). Polymerase chain reaction (PCR) amplifications were performed in a final volume of 10 µL, which contained 1 µL of extracted DNA, 1 µL of 25 mM $MgCl_2$, 0.1 µL of 10 mM dNTPs, 1 µL of PCR buffer (Eurogentec), 1 unit of *Taq* DNA polymerase (Eurogentec Red Goldstar), 0.5 µL of forward primer, and 0.5 µL of reverse primer. PCRs were performed separately for each primer pair on a PTC-200 thermocycler using the following cycling conditions: initial denaturation at 92 °C for 1 min 30 s; 30 cycles of 92 °C for 30 s, annealing temperature for 45 s, 72 °C for 1 min 30 s; and final elongation at 72 °C for 10 min. Annealing temperatures were, respectively, 55 °C, 45 °C, and 55 °C for *12s rRNA*, *16s rRNA*, and *COI*. The method of Sanger (1981) was carried out using Applied Biosystems BygDye protocol, and the sequence of 1580 nucleotides for each individual was obtained. Products of the sequencing reactions were then analysed on an ABI Prism 310 sequencer. Chromatograms were manually corrected using Chromas 2.23 (Technelysium Pty. Ltd), and sequences were aligned using CLUSTALW 1.83 (Thompson *et al*. 1994). Phylogenetic trees were reconstructed by a tree-bisection-reconnection (TBR) branch-swapping-algorithm, under a general time reversible (GTR) model, using a maximum-likelihood algorithm with the following parameters under PAUP* 4.0.10 (Swofford 2002): eight evolutionary rate categories, gamma shape parameter (alpha) estimated, no invariable sites. Bootstrap values were calculated on 100 replications. Likelihoods of constrained and nonconstrained trees were compared with a Kishino–Hasegawa (RELL bootstrap) test, using PAUP*. The molecular clock was tested by a likelihood ratio test comparing likelihoods obtained by the two PHYLIP 3.6b package programs (Felsenstein 2004), DNAML and DNAMLK (less time-consuming than under PAUP*), using the parameters previously estimated by PAUP*, and eight evolutionary rate categories (including invariant sites). Sequence diversities and distances between individual groups were calculated using gamma nucleotide

(TN93) and gamma amino acid distance models under MEGA 2.1 (Kumar *et al*. 2001).

Microsatellite markers

In order to compare diversities and historical biogeography of *A. obtectus* individuals, we amplified individuals whose mtDNA sequences had been examined earlier, with five microsatellite loci (*C12*, *E01*, *E07*, *F09*, and *G08*) defined by Alvarez *et al*. (2004b). We genotyped other samples (when available) collected in each site, plus samples proceeding from four populations geographically close to SJS (MAL, TEJ, TEP, STM) and one population close to COE (SIC). In all, we genotyped 191 individuals distributed as follows: 102 Mexican individuals [COE (11), MAL (12), SAG (6), SCA (1), SFP (1), SIC (3), SJS (10), STM (16), STO (1), TEJ (6), TEP (14), TLA (3), XOT (17), YOH (1)]; 52 Old World individuals [Cameroon (1), France (21, in two sites within 80 km), Spain (19), Switzerland (11)]; 37 Peruvian individuals, all of them proceeding from Mamorimamba. Total genomic DNA was extracted using DNeasy Kit (QIAGEN). PCR amplifications were performed according to Alvarez *et al*. (2004b). Products of the reactions were then analysed on ABI Prism 310 and 3100 sequencers. Genotypes were analysed using GENESCAN 3.1.2 and GENOTYPER 2.5 software (Applied Biosystems). Exact sizes of alleles were manually corrected to round integer sizes. Allelic richnesses for the three biogeographic groups (Mexico, Peru, and Old World) were calculated using FSTAT 2.9.3 (Goudet 1995). Pairwise genetic differentiation between the 191 genotyped individuals was calculated using GENEPOP 3.4 (Raymond & Rousset 1995) by computing estimates of a genetic parameter analogous to estimates of $F_{ST}/(1 - F_{ST})$ for populations (Rousset 2000). The dendrogram was built from the matrix of genetic distance using the NEIGHBOUR program from the PHYLIP package (Felsenstein 2004).

Results

Mitochondrial DNA

Sequencing of *12s rRNA*, *16s rRNA*, and *COI* revealed polymorphism among individuals. For *12s rRNA*, only two haplotypes were found: one for New World individuals, and the other for Old World individuals plus the Mexican individual originating from the XOT site (Accession nos AY826433–AY826457). For *16s rRNA*, individuals were distributed among four haplotypes (see table 2 Accession nos AY826458–AY826482). The first haplotype was shared by almost all Mexican individuals (except those coming from sites XOT and YOH); a second haplotype was found only in the Peruvian individuals and in one Mexican

Table 2 Distribution of the four *16s rRNA* haplotypes among the sequenced individuals, showing only polymorphic sections, and with variant sites in bold and underlined

Haplotype		10 17 |......|		21 27 |.....|		186 192 |.....|		307 313 |.....|
1	Peru MAM 2	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 6	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 8	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 12	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 13	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 15	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 16	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 19	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 23	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 29	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Mexico YOH	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
2	Mexico SAG 5	TATTCCCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
	Mexico TLA 1	TATTCCCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
	Mexico SCA	TATTCCCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
	Mexico SFP	TATTCCCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
	Mexico STO	TATTCCCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
	Mexico COE 2	TATTCCCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
	Mexico SJS 2	TATTCCCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
3	Spain PRA 1	TATT**T**CCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
	Mexico XOT 3	TATT**T**CCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
4	Switzerland NEU 1	TATT**T**CCA	*	TCTGCAC	*	TTC**G**ATT	*	AAT**C**TTA
	Cameroon YAH	TATT**T**CCA	*	TCTGCAC	*	TTC**G**ATT	*	AAT**C**TTA
	France CHA 5	TATT**T**CCA	*	TCTGCAC	*	TTC**G**ATT	*	AAT**C**TTA

individual originating from YOH; the third haplotype was present in the Spanish individual and the Mexican individual originating from XOT; and the fourth haplotype was present only in the individuals from Switzerland, Cameroon, and France. For *COI*, almost each individual exhibited a unique haplotype (Accession nos AY826483–AY826507).

The tree reconstructed for *Acanthoscelides obtectus* — anchored by its two closest relatives *Acanthoscelides obvelatus* and *Acanthoscelides argillaceus* — clearly showed the basal position of a small clade comprising the 10 Peruvian individuals and one of the Mexican individuals (YOH) (see Fig. 1a). This position is shown not only after the common analysis of all nucleotides sequenced, but also by the individual analysis of each of the two most variable genes, *16s rRNA* and *COI*. This congruence strengthens the basal phylogeographic position of the Peruvian clade. A Kishino–Hasegawa test comparing the likelihood of this tree with the likelihood of a tree constrained to have the Peruvian clade in nonbasal position, found a trend in the same direction ($P = 0.10$).

From this first clade branches an unresolved cluster — with only Mexican individuals — in which is nested another clade with mostly Old World individuals [but one Mexican individual (XOT)]. The monophyly of this last clade is supported by the individual analysis of each of the three genes. Moreover, constraining the individual originating from XOT to be in the larger group of taxa leads to a tree whose likelihood is significantly lower (Kishino–Hasegawa test, $P = 0.038$). Using the same method, an analogous tendency showing a lower tree likelihood for the constrained topology was also obtained by constraining the individual originating from YOH to be in the larger group of Mexican taxa ($P = 0.09$). Topologies obtained using DNAML and DNAMLK without outgroups were identical for all major clades, and values for ln (likelihood) were, respectively, –2342.37865 and –2325.38982. The likelihood ratio test is passed and the molecular clock cannot be rejected ($P = 0.0655$).

COI haplotype diversities calculated with a nucleotide gamma distance (TN93) were 0.0066 ± 0.0013 and 0.0116 ± 0.0021 for *A. obtectus* and *A. obvelatus*, respectively (ratio = 0.57). Diversities calculated with amino acid gamma distance are 0.0053 ± 0.0025 and 0.0189 ± 0.0051, respectively (ratio = 0.28). Sequence diversity in *A. obtectus* was thus much lower than in *A. obvelatus*, about 43% lower for nucleotides and 72% lower for amino acids.

Microsatellite markers

Four of the five examined microsatellite loci (*C12*, *E07*, *F09*, and *G08*) amplified in all 191 individuals, and were polymorphic. The fifth locus *E01*, however, gave much poorer results — especially in Peruvian individuals in which fewer than 50% of individuals were amplified — and we decided to discard it. An online supplement (see supplementary material) containing the allelic data (PCR product sizes) for loci *C12*, *E07*, *F09*, and *G08* is provided as an Excel file. Allelic richness for the four loci retained, in each site where at least 10 individuals had been genotyped, ranged from 3.5735 and 7.6153 and did not vary significantly among sites ($P = 0.068$). When considering three biogeographical regions (i.e. Old World, Mexico, Peru), allelic richnesses were, respectively, 4.009, 6.879, and 5.215. Whereas allelic richness was not significantly different between Mexico and Peru ($P = 0.2073$), differences appeared significant when comparing the Old World with both Mexico ($P = 0.017$) and Peru ($P = 0.0328$).

The unrooted neighbour-joining (NJ) tree reconstructed from the distance matrix for the 191 genotypes is represented in Fig. 1(b). It shows that most of the Peruvian individuals (20 of 37) are grouped in one well-separated clade (A), which includes only one non-Peruvian individual, one from the Mexican population YOH. Several individuals of this clade demonstrate much longer branch lengths than any other individual from other sites. The other 170 individuals (which include 17 Peruvian individuals) are grouped in three clades and demonstrate much shorter branches. Clade B comprises 14 Peruvian individuals and 16 Mexican individuals. Clade C contains mostly European individuals (51), along with 3 Peruvian and 18 Mexican individuals. Clade D includes 67 Mexican individuals, and one Old World individual from the Spanish population.

Discussion

Although bootstrap values were not exceptionally high in the mtDNA analysis (ranging from 53 to 78 for the three major nodes), all sequenced genes gave congruent information, and our results thus appear robust. The most parsimonious scenario places the Peruvian individuals at a basal position, along with one Mexican individual (YOH). Because the Mexican individual from YOH emerged from an extensive culture of a modern bean variety (the worldwide distributed 'negro mata' variety) in Puebla State, and because the Peruvian individuals come from a traditionally managed field of landraces in a remote valley of the Departamento de Cajamarca where incoming long-distance human-mediated migrations seem improbable, it is more likely that the Mexican individual of this basal clade originated from a recent migrant from South America (through human trade of broadly commercialized beans).

This Andean origin is further supported by the microsatellite analysis, despite the fact that allelic richness was not greater in Peru than in Mexico. What distinguishes several Peruvian individuals is that they are on much longer branches than individuals from Mexico and Europe. Long branches of the Peruvian individuals signify that these individuals are issued from older lineages than the other individuals, all on short branches. Longer branches

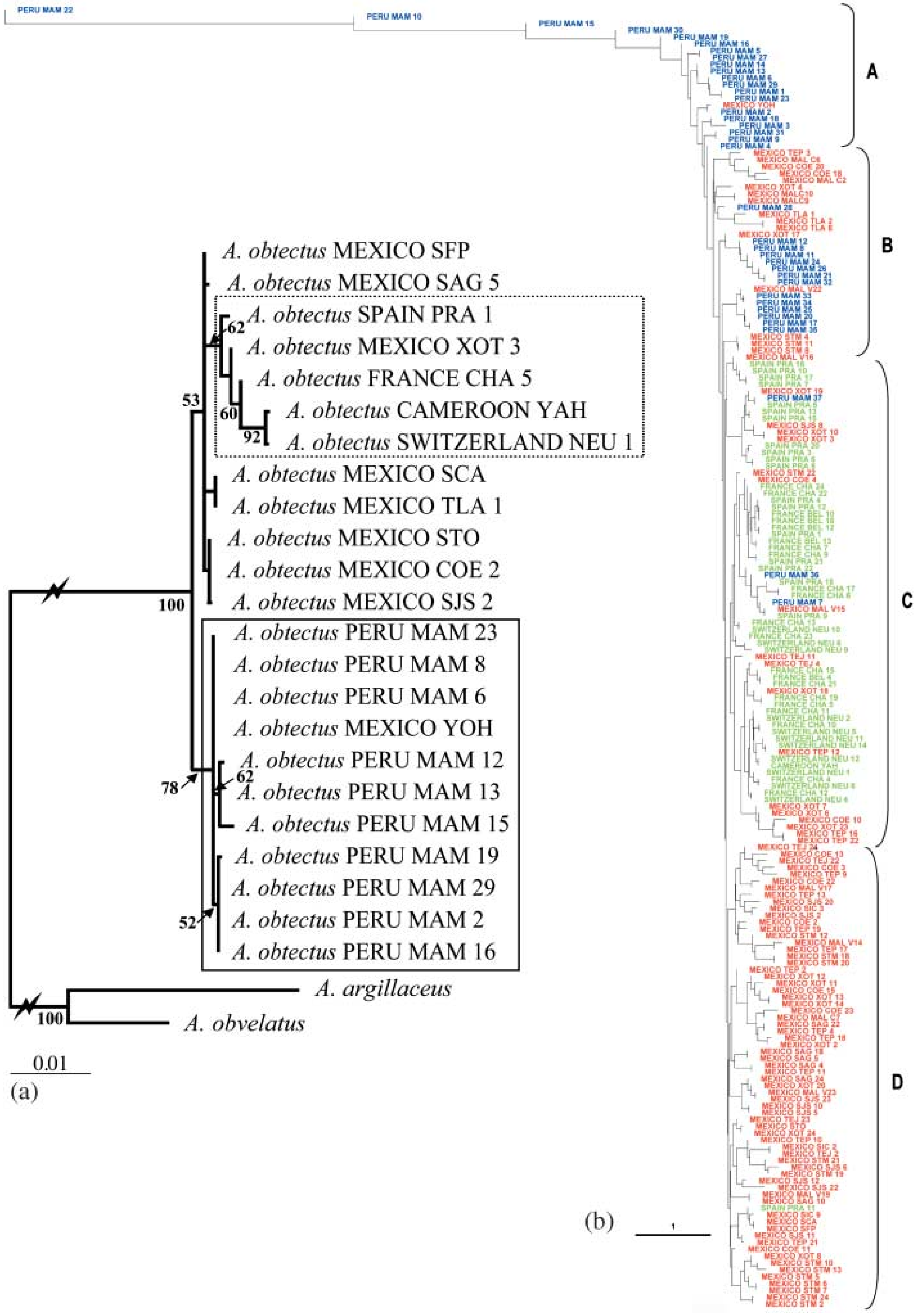

Fig. 1 (a) Phylogenetic reconstruction of individuals sequenced for the three genes *12s rRNA*, *16s rRNA*, and *COI*. Numbers at the nodes show bootstrap values. The frame in the continuous line illustrates the basal Peruvian clade. The frame in the dotted line illustrates the Old World clade. The ⫽ indicates that the very long branches linking *Acanthoscelides obtectus* individuals to outgroups are condensed by 0.1. (b) Dendrogram of 191 individuals genotyped for four microsatellite loci. Individuals in blue are from Peru, individuals in red are from Mexico, and individuals in green are from the Old World.

could also be explained by hybridization of *Acanthoscelides obtectus* with another bruchid species in this area. However, the microsatellite loci used in this study are all species-specific, and have not been able to cross-amplify any other species, including those most closely related to *A. obtectus* (*Acanthoscelides argillaceus* and *Acanthoscelides obvelatus*). Furthermore, we have never seen evidence of interspecific hybridization in any bruchis, even between sister species such as *A. obtectus* and *A. obvelatus* (Alvarez *et al*. 2004a). We thus prefer to consider much more likely the hypothesis that long branches in Peru reflect older lineages. Furthermore, despite the fact that a majority of genotyped individuals from Peru branch together on the tree, some Peruvian individuals are found in two other clades. They could thus have included the ancestral members of all clades of the phylogenetic tree. Concerning the YOH individual, which was the sole Mexican individual to group with the Peruvian individuals in the mtDNA analysis, it also branches in the main Peruvian clade in the microsatellite analysis, confirming its proximity to Peruvian individuals. However, because we had sampled only one individual in YOH, we could not examine if other individuals would have branched elsewhere. Nevertheless, as in the mtDNA analysis, the other clades containing Mexican individuals include all five that were found on wild bean populations. The most likely hypothesis to account for our results is that the YOH individual derives from a recent introduction of *A. obtectus* from Peru (or elsewhere in the species' South American range), associated with the intensive cultivation of a widespread commercial bean variety. The alternative hypothesis, that the Mexican individual from the YOH site is more closely related to Mexican individuals than to the Peruvian individual, the latter having originated from a recent migration from Mexico, seems unlikely. Moreover, branch length of the YOH individual in the microsatellite analysis is relatively short, in comparison to several Peruvian individuals.

In the mtDNA analysis, Old World individuals appear to have originated from a limited genetic base and are of Mexican ancestry. The microsatellite analysis, however, does not confirm the monophyly of Old World individuals, probably because of shared ancestral polymorphism in individuals from Mexico and from the Old World, and also because the method used to calculate genetic distances between individuals (Rousset 2000) is more appropriate to assess distance in theoretical continuous populations. However, in this analysis, Old World individuals are more closely related to Mexican individuals than to most Peruvian individuals. The XOT individual, which branches together with Old World individuals in the mtDNA analysis, could have resulted from a human-mediated migration back to Mexico from a population of European origin. Alternatively, it might be close to the Mexican population that gave rise to the migrants that reached the Old World. Examination of microsatellite results provides a clue to the polarity of the migration. Whereas Old World individuals are well segregated [51 individuals of 52 branch into the same clade (C)], individuals from XOT are relatively diverse and are present in three of the four clades (Fig. 1b). A migration from Europe back to Mexico would probably have involved very few individuals, with a very low genetic diversity. It is much more parsimonious to consider that individuals from XOT are closely related to the ancestors of the Old World population, which resulted from migration 500 BP.

Ancient history of A. obtectus

According to current hypotheses, *A. obtectus* lost its capacity for diapause subsequent to domestication of *Phaseolus vulgaris*. The evolution of multivoltinism was adaptive because it allowed continuous reproduction in granaries. However, this hypothesis no longer seems tenable. First, both *A. obtectus* and its sister species *A. obvelatus* occur on both wild and domesticated beans, and only the difference in voltinism is characteristic of the species. Whether attacking wild or domesticated beans, *A. obtectus* is multivoltine and *A. obvelatus* is univoltine (N. Alvarez, unpublished). Second, we have recently shown (Alvarez *et al*. 2004a) that these two species diverged at least several million years ago. Therefore, assuming that the divergence of the voltinism character is as old as the divergence between the species, this is incompatible with a scenario of evolution of multivoltinism in *A. obtectus* after domestication of *P. vulgaris* (*c*. 7000 BP).

Multivoltinism thus appears to have characterized *A. obtectus* prior to bean domestication. Nonetheless, this trait would have made its survival impossible in Mexican wild bean populations, which show annual and strongly seasonal production of a single fruit crop (in the dry season). Wild bean populations in Mexico appear to be sinks for *A. obtectus* (Alvarez *et al*. submitted). However, in other parts of its Neotropical range, *A. obtectus* encounters potential host plants with phenologies that could be effectively exploited by a multivoltine bruchid. For example, spontaneous forms (escapees from cultivation) of domesticated *Phaseolus polyanthus* Greenman at intermediate altitudes (1000–2600 m) in the equatorial Andes are known to fruit throughout the year (A. Delgado-Salinas, personal communication) This character is of real importance, since Andean farmers call this bean species 'frijol de toda la vida', which means 'bean of the whole life'. The distribution of *P. polyanthus* extends from Peru to Mexico; wild forms are known today only from mountains of Guatemala (Schmitt & Debouck 1991), but may have been much more broadly distributed prior to the domestication of this species. If *A. obtectus* were associated with this or another wild *Phaseolus* with continuous fruiting, the capacity to

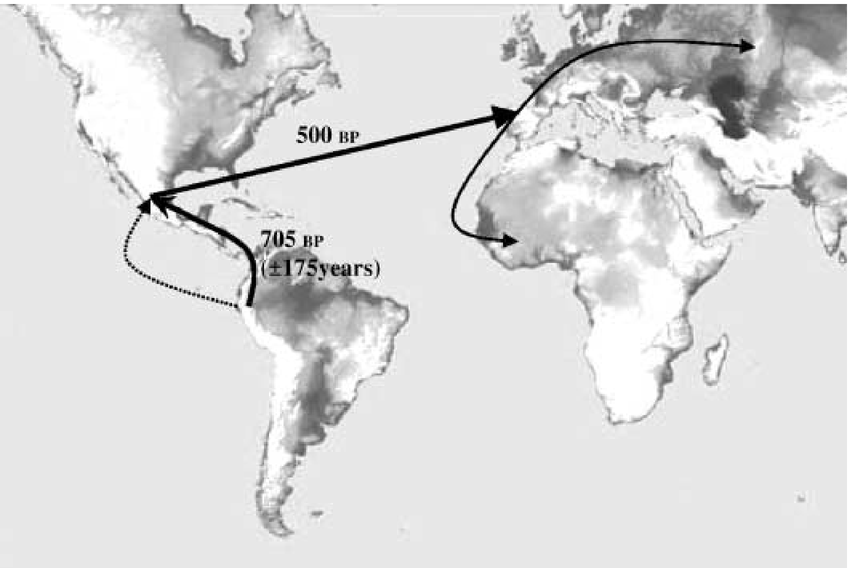

Fig. 2 Hypothetical pathways of the main colonization of *Acanthoscelides obtectus* from Andean America to the rest of the world by human-mediated migrations. Values on the arrows are in years before present. The arrow in dotted line illustrates a second, more recent migration in the same direction, from Peru to Mexico.

produce multiple (5–10) generations per year, rather than one, would have been strongly favoured by selection. Nowadays, *A. obtectus* is known to develop on *P. polyanthus* throughout the range of this bean species.

The trees based on our mtDNA and microsatellite evidence show that this scenario is plausible. The Peruvian individuals occupy the most basal position in the clade in the mtDNA tree, whereas in the microsatellite analysis, branch lengths of Peruvian individuals are much longer than those of other individuals. Peruvian individuals originated from a remote valley of the Departamento de Cajamarca, and it is unlikely that bruchids in such a remote area are recent migrants from Mexico. Moreover, *P. polyanthus* fruits continuously all around the sampling station (A. Delgado-Salinas, personal communication), because valleys of the tropical Andes show little seasonality (Brush 1982).

We propose a new hypothesis that is consistent with biological information and molecular evidence. According to this hypothesis, multivoltinism characterized *A. obtectus* before bean domestication. *A. obtectus* may have been originally associated with wild beans (one or more species) that individually or collectively provided fruits year-round. Such an ecological situation is most likely to be encountered in equatorial regions at mid-altitude with little seasonality. However, the multivoltinism of this species gave it an advantage, compared to univoltine species, in the exploitation of beans stored in granaries, enabling *A. obtectus* and not other species to become a serious pest of domesticated beans. *Phaseolus vulgaris* was domesticated twice independently, in Andean America and Mesoamerica (Gepts & Papa 2002; Islam *et al*. 2002), and we propose that the association of *A. obtectus* with domesticated beans may have originated in the first of these two centres of domestication, followed by the spread of *A. obtectus* to Mesoamerica. Parallel with this hypothesis, we propose that *A. obvelatus* originated in Mesoamerica and became associated with domesticated beans in this second centre of domestication.

Consistent with this scenario are the data obtained by comparing sequence diversities between *A. obtectus* and its sister species *A. obvelatus*, which occupy a very similar niche. Greater genetic diversity in an area may indicate the long presence of a species in the area; diversity should be reduced if populations were initiated from a reduced pool of migrants that colonized a new region. Our results on *COI*, which demonstrate diversities in Mexico ranging from 1.75 to 3.57 times greater in *A. obvelatus* than in *A. obtectus*, suggest that *A. obtectus* may have arrived relatively recently in Mexico. The extent of genetic diversity of *A. obtectus* populations in South America is still unknown.

Recent history of A. obtectus

Based on the introduction of beans into Europe in the early 16th century (Sturtevant 1887), the Old World clade in the mtDNA tree can be considered to have emerged about 500 BP through one sole event from the undifferentiated clade of Mexican individuals in which it is nested. As the mean divergence (calculated with a TN93 NJ model) between the two groups is 0.0051 nucleotide, we can calibrate a molecular clock of $1.02 * 10^{-5}$ mutations/nucleotide/year. Thus, as the divergence between the basal clade and the other clades is 0.0094 ± 0.0021, we can situate the arrival of *A. obtectus* in Mexico at 705 ± 175 BP (Fig. 2). This date appears to be relatively recent. However, such utilization of the molecular clock must be done cautiously because neutral diversity in populations remains a stochastic process, especially when considering closely related sequences, and because we don't know if the diversity present in Old World individuals is still present in Mexico (i.e. Old World and Mexican populations could share ancestral polymorphism). Nevertheless, we can suppose a quite recent migration of *A. obtectus* from South America to Mexico. This is corroborated by a recent study on the ecology of the two sibling species (Alvarez *et al*. submitted) which showed that *A. obvelatus* is almost always more frequent than *A. obtectus* in about 50 sampled sites of the Mexican altiplano. This suggests that *A. obvelatus* is more adapted to the environmental conditions of the Mexican altiplano than is *A. obtectus*, and that the presence of *A. obvelatus* in Mexico is older than that of *A. obtectus*.

A recent arrival of *A. obtectus* in Mexico could be the consequence of the tropical climate of lowland Central America, which should act as an important barrier to this species. Indeed, even today its densities at low altitudes are much lower than those of other bruchid species, such as *Zabrotes subfasciatus* Boheman. Barriers such as the low-lying Isthmus of Panama could have prevented *A. obtectus* from the Andes from reaching the Central American or Mexican highlands. In such lowlands, *A. obtectus* would probably have been supplanted by other bruchid species (Leroi *et al*. 1990). We postulate that *A. obtectus* arrived in Mexico by recent long-range migration through exchanges of bean seeds, which may have occurred once the web of human communication was sufficiently developed in the Americas. In this context, the date 700 BP — i.e. about 1300 AD — is plausible, given the archaeological records showing agricultural exchanges between Mesoamerican and Andean civilizations, for example between the years 1000 and 1525 (Pacheco 2000). The possibility that more ancient trade between the two civilizations occurred is also suggested by several authors (e.g. Webb 1974; Lorenzo 2000). However, since precaution is required when applying a molecular clock calibrated on a very recent event, we cannot yet discard the hypothesis that *A. obtectus* could have migrated on its own, independently of human-mediated seed movements, long before bean domestication. Indeed, climatic barriers in Central America seem not to have been an obstacle for wild beans such as *P. vulgaris*, which is not particularly adapted to low altitudes.

Nonetheless, our results give credence to the hypothesis that long-distance exchanges occurred between Mesoamerica and Andean America posterior to bean domestication. Movement of beans could have been the way by which *A. obtectus* migrated to Mexico. Consistent with this hypothesis, ancient gene flow between the Mesoamerican and Andean centres of origin of cultivated beans has been shown in several landraces (Gepts 1998).

Another remarkable finding is that the arrival of *A. obtectus* in the Old World seems to have resulted from a single colonization event, probably (based on the mtDNA tree) starting in Spain. The hypothesis of a single colonization is supported by the reciprocal proximity of mtDNA sequences of Old World individuals. Data from analysis of microsatellite markers confirm this probable origin, because the only Old World individual that branches outside clade B is a Spanish individual, which branches along with Mexican individuals of clade D (Fig. 1b). Because all Old World individuals but one branch together in the microsatellite analysis, a sole colonization event from Mexico, rather than a hypothesis of multiple colonization, seems more likely. However, it cannot be excluded that other colonization events have contributed (rarely) to Old World populations. The single Spanish individual branching outside the Old World group in the microsatellite analysis may represent the influence of such secondary, minor colonization, or alternatively, it may represent ancestral polymorphism. This is again consistent with historical evidence of bean diffusion. The capacity to disperse through human-mediated migrations and further adapt to new ecological conditions may explain why *A. obtectus* is today a pest of at least six legume genera (Johnson 1983, 1990). Attention concerning its biology and natural enemies has so far been focused on Mesoamerica. However, we have known for some time that domestication of beans has a complex history, with two independent centres of domestication. This study points to the interest — and the urgent need — of devoting more attention to the Andean American centre, where this bruchid seems to have originated, in particular concerning efforts to find appropriate parasitoid species for biological control. The first step to reach this goal should be increased sampling of South American populations, not only for phylogeographical, but also for ecological studies.

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This work is part of a project led by Betty Benrey on the effects of plant domestication on phytophagous insects and their parasitoïds. Nadir Alvarez works on legume/bruchid interactions, focusing on the Neotropical genus *Acanthoscelides* (Coleoptera: Bruchidae). Doyle McKey and Martine Hossaert-McKey are investigating the evolutionary ecology of several plant/insect systems. Céline Born works on population genetics of several animal and plant species. Lény Mercier's interests are centred on ecological interactions between insects and plants.
