Analysis of *Rhizobium etli* and of its symbiosis with wild *Phaseolus vulgaris* supports coevolution in centers of host diversification

O. Mario Aguilar*, Omar Riva, and Eitel Peltzer

Instituto de Bioquímica y Biología Molecular, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 1900 La Plata, Argentina

Communicated by James M. Tiedje, Michigan State University, East Lansing, MI, July 23, 2004 (received for review February 19, 2004)

Common beans (Phaseolus vulgaris) comprise three major geographic genetic pools, one in Mexico, Central America, and Colombia, another in the southern Andes, and a third in Ecuador and northern Peru. Species Rhizobium etli is the predominant rhizobia found symbiotically associated with beans in the Americas. We have found polymorphism in the common nodulation gene nodC among R. etli strains from a wide range of geographical origins, which disclosed three nodC types. The different nodC alleles in American strains show varying predominance in their regional distributions in correlation with the centers of bean genetic diversification (BD centers). By cross-inoculating wild common beans from the three BD centers with soils from Mexico, Ecuador, Bolivia, and Northwestern Argentina, the R. etli populations from nodules originated from Mexican soil again showed allele predominance that was opposite to those originated from Bolivian and Argentinean soil, whereas populations from Ecuadorian soil were intermediate. These results also indicated that the preferential nodulation of beans by geographically related R. etli lineages was independent of the nodulating environment. Coinoculation of wild common beans from each of the three BD centers with an equicellular mixture of R. etli strains representative of the Mesoamerican and southern Andean lineages revealed a host-dependent distinct competitiveness: beans from the Mesoamerican genetic pool were almost exclusively nodulated by strains from their host region, whereas nodules of beans from the southern Andes were largely occupied by the geographically cognate R. etli lineages. These results suggest coevolution in the centers of host genetic diversification.

Phaseolus vulgaris-rhizobia coevolution | *Rhizobium etli* lineages | symbiotic interaction | *nod*C gene

t is generally accepted that *Phaseolus vulgaris* L. (the common bean) is native to the Americas. The analysis of natural bean populations, based on diverse criteria such as morphological traits, electrophoresis, and genetic typing of phaseolin, isozymes, restriction fragment length polymorphism (RFLP), and amplified RFLP, has led to the proposal of two major distinguishable areas or centers of bean genetic diversification (BD centers): the Mesoamerican center (Mexico, Central America, and Colombia) and the Andean center in South America (Ecuador, Peru, and Argentina) (1-4). Analysis of the multigenic family coding for phaseolin led Kami et al. (5) to speculate that the ancestral wild P. vulgaris type may be represented by the wild common bean populations from Ecuador and northern Peru and to propose the occurrence of an intermediate gene pool. Compatible with this view, Geffroy et al. (6) more recently investigated anthracnoseresistance specificities in genotypes from the Mesoamerican and Andean gene pools and identified a gene cluster with both Andean and Mesoamerican host-resistance specificities, suggesting that the locus existed before the separation of the two major gene pools of P. vulgaris. However, their results indicated that further coevolution between the pathogen agent and bean resulted in a differentiation for resistance in the three centers of diversification of the host (6).

Soil bacteria of the genus *Rhizobium* are able to induce nitrogenfixing symbiotic association with the roots of leguminous plants. *P. vulgaris* is a promiscuous legume that forms nodules with a diversity of rhizobial genotypes. To date, five *Rhizobium* species have been recognized as microsymbionts of *P. vulgaris: Rhizobium etli* bv. phaseoli, *Rhizobium leguminosarum* bv. phaseoli, *Rhizobium tropici*, *Rhizobium gallicum*, and *Rhizobium giardini* (7). *R. etli* bv. phaseoli is the predominant species found associated with both wild and cultivated common beans from Mexico, Colombia, and the southern Andes (7), leading to the proposal of coevolution between bean host and rhizobia, although this has not been fully demonstrated and remains a matter of conjecture (8, 9).

Because common beans have become an important crop worldwide, the dissemination of microorganisms harbored by the plants and seeds during the process of export to new regions may have occurred as well. Thus, *R. etli* is found in regions where beans have been introduced since as early as the 16th century, after the discovery of the Americas. *R. etli* has been isolated from nodulated, cultivated beans in Spain, France, Austria, Kenya, Tunisia, and Indonesia (7, 10–12).

Species *R. etli* predominates in nodules formed in wild common beans in northwestern Argentina (NWA); however, population diversity was revealed at the level of sampling, within plants and sites of collection, by using plasmid profile and DNA fingerprinting (13).

More recently, we found the common nodulation gene *nod*C to be polymorphic among *R. etli* populations from NWA (14). In this study, we have explored the usefulness of *nod*C typing as molecular marker to characterize *R. etli* populations of diverse geographical origin and have assessed its value as an indicator of coevolution between rhizobia and wild beans in the Americas. Toward this aim we first examined the distribution of *nod*C types among *R. etli* populations from NWA and also among *R. etli* strains collected in the three BD centers. Second, rhizobial populations retrieved from soil samples from the same three BD centers by using wild and cultivated common beans as trapping host were *nod*C-typed to characterize native bean rhizobia. Finally, a set of wild and cultivated accessions representative of the Mesoamerican and Andean groups was analyzed in coinoculation assays for interactions with strains representative of the *nod*C types found among *R. etli* isolates.

Methods

Rhizobial Strains. Rhizobia examined in this work were isolated from nodules of wild common beans growing in NWA or were retrieved in the laboratory from field soils. From each plant sampled, three to six nodules were randomly excised and surface-sterilized with ethanol and hydrogen peroxide. Rhizobia were isolated axenically on YEM-Congo red agar medium as described by Vincent (15). Species assignment of bean isolates was done by both the 16S

Abbreviations: BD center, center of bean genetic diversification; NWA, northwestern Argentina; RFLP, restriction fragment length polymorphism.

^{*}To whom correspondence should be addressed. E-mail: aguilar@biol.unlp.edu.ar.

^{© 2004} by The National Academy of Sciences of the USA

rRNA-encoding DNA–RFLP analysis and the *nif*H PCR screening procedure as described in ref. 13. Almost all isolates from wild and common bean varieties were found to belong to species *R. etli*; only very few were characterized as *R. leguminosarum* by. phaseoli.

Plant Material. Seeds of natural populations of wild bean plants from NWA were provided by Roberto Neumann [Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Salta, Salta, Argentina]. Seeds of 30 wild bean accessions chosen to represent gene pools on the basis of previous results were provided by Daniel Debouck (Unidad de Recursos Genéticos, Centro Internacional de Agricultura Tropical, Cali, Colombia). Seeds of the black bean cultivar Nag12 and the white bean cultivar Alubia Cerrillos were provided by Susana García Medina (INTA, Estación Experimental Agropecuaria Salta), seeds of the Brazilian cultivar Aporé were provided by Mariangela Hungría (Empresa Brasileira de Pesquisa Agropecuaria-Soja, Londrina, Brazil), and seeds of the Mexican cultivar Negro Xamapa were provided by Esperanza Martínez-Romero (Centro de Fijación de Nitrógeno, Cuernavaca, Mexico).

DNA Isolation and RFLP Analysis of PCR-Amplified Symbiotic Genes.

DNA from rhizobial cells grown in YEM medium was prepared with a rapid method as described by Alippi and Aguilar (16) and was used as a template in PCR reactions. *nod*C PCR amplification was performed as described in ref. 17. The primers used for *nod*A PCR amplification were deduced from the published *R. etli nod*A sequence (GenBank accession no. M58625), and reaction conditions were identical to those used for *nod*C PCR. Oligonucleotides *nod*Af (5'-CGGTGGAAAGTGTGCTGGCAAACGA-3') and *nod*Ar (5'-ATCCGGGGAACCTTGATCGAGCGGAAT-3') were used as forward and reverse primers, respectively, to amplify ~560 bp of the 588 bp of the *nod*A gene. Aliquots of the PCR product were used for restriction pattern analysis with endonucleases *AluI*, *MspI*, *BcII*, *HaeIII*, *DdeI*, *MboI*, *CfoI*, *RsaI*, *HinfI*, and *TaqI* (*nod*A) and *HinfI*, *CfoI*, *MspI*, *NdeI*, *RsaI*, and *HaeIII* (*nod*C), respectively. The RFLP analysis was performed by electrophoresis in 2% agarose gel.

Plant Inoculation with Soil Samples. Soil isolates were recovered from nodules of wild and cultivated beans, which were grown in the laboratory after inoculation with soil suspensions prepared with soil samples. Soil samples were obtained from different field sites from Mexico, Ecuador, Bolivia, and NWA and were provided, respectively, by Jesús Caballero Mellado (Centro de Fijación de Nitrógeno), Gustavo Bernal (Instituto Nacional Autónomo de Investigaciones Agropecuarias, Estación Experimental Santa Catalina, Santa Catalina, Ecuador), Irene Christiansen (Programa Andino Extensivo en Cultivos Hortícola, Santa Cruz de la Sierra, Bolivia), and Mario Chocobar (INTA, Estación Experimental Agropecuaria Salta). One hundred grams of soil was suspended in 1 liter of sterile distilled water, and 8 ml of this suspension per plant was used for inoculation. Plant assays were performed with seeds that were surface-sterilized sequentially with 75% ethanol for 1 min and sodium hypochlorite for 6 min and finally washed with sterile water. Germinated seedlings inoculated with soil suspensions were grown in 300-ml plastic pots filled with sterilized vermiculite and were watered twice with N-free mineral nutrient solution (15) and with sterile distilled water as required.

Coinoculation Experiments. Cultures of *R. etli* strains showing *nod*C type α and type δ were grown separately to late exponential phase in YEM broth. Rhizobial mixtures of strains *nod*C type α and δ (ratio 1:1) were made, and 10 ml of this mixture, containing 10⁸ bacteria, was applied per plant. Mixtures assayed were CFNX5 (isolated in Mexico) plus 55N1 (isolated in NWA), SC15 (isolated in NWA) plus 55N1, and SC15 plus 90N3 (isolated in NWA). At least two plants of each bean accession or cultivar were inoculated with each mixture. In each experiment, a set of plants of both



Fig. 1. Representative RFLP patterns of *nod*C types of *R. etli* isolates from the Americas. Shown are the results of *HinfL* digestion of PCR-amplified *nod*C gene. MWM, molecular weight marker (100 bp, Promega).

Mesoamerican and south Andean origins was treated in parallel with the same inoculant mixture. Plants were incubated at $26-28^{\circ}$ C in a day/night cycle of 16/8 h, watered as described above, and harvested ≈ 25 days after inoculation. All nodules from each plant were excised, or, alternatively, in cases of numerous nodulation, only nodules located in the upper part of the principal root were removed for further analysis. Nodules were individually surface-sterilized with 96% ethanol and hydrogen peroxide and were crushed, and rhizobia were axenically isolated in YEM-Congo red medium. About 25 nodules per plant and two plants from each bean variety were examined for *nod*C profile.

Statistics. All data were subjected to variance analysis with Student's t test (18).

Results

RFLP Analysis of PCR-Amplified Symbiotic Genes nodA and nodC in R. etli Isolates from NWA. One hundred and sixty-four R. etli isolates were obtained from nodules of 51 plants of P. vulgaris var. aborigineus collected from 16 different sites in NWA. These were found in virgin lands having no records of previous agricultural management. The common nodulation genes *nod*A and *nod*C of each isolate were amplified and analyzed by RFLP analysis. The nodA RFLP patterns were found to be identical in all of the isolates, as well as in the type strain R. etli CFN42. In contrast, RFLP analysis of the *nod*C PCR product resulted in three distinct patterns that, for convenience, were designated as *nod*C types α , γ , and δ (Fig. 1). In addition, another distinct nodC pattern was found only in isolates of wild common beans carrying the R. leguminosarum-like allele that codes for the 16S rRNA gene (pattern *nod*C type β in Fig. 1). These results were in agreement with those from our previous work on indigenous populations isolated from common bean nodules collected in NWA (14). The type strain R. etli CFN 42 showed nodC type α . Because the sole use of only endonuclease *Hin*fI sufficed to yield the same four distinguishable nodC types, hereafter the designation "nodC types" will refer to the patterns resulting from HinfI restriction of the nodC PCR-amplified fragment.

The three *nod*C types α , γ , and δ were detected among the *R. etli* isolates; however, type δ was found to be the most prevalent, occurring in 132 of 164 isolates examined, which represents a probability of 0.80 \pm 0.05 at a *P* = 0.05 level of confidence. The remaining 32 isolates were distributed among types α (*n* = 24) and γ (*n* = 8). In our analysis of the *nod*C gene, we also assayed another collection of isolates of wild common beans from NWA that had been previously characterized by Aguilar *et al.* (13). Thirteen of 15 isolates having the *R. etli* 16S rRNA allele exhibited *nod*C type δ , whereas the other two isolates exhibited *nod*C type α . The four

isolates of collection having the *R. leguminosarum* 16S rRNA allele exhibited types β (n = 1) and δ (n = 3) (data not shown). These results demonstrated that allele *nod*C type δ is highly represented among rhizobia isolated from wild common bean nodules of NWA.

Distribution of nodC Alleles in a Worldwide Collection of R. etli Strains.

To determine the geographical distribution of nodC types, a collection of 94 R. etli strains from diverse geographical origins other than NWA was assayed. These strains are examples of populations from the United States (n = 5), Mexico (n = 21), Belize (n = 2), Colombia (n = 10), Ecuador (n = 16), Peru (n = 16)2), Brazil (n = 12), Spain (n = 21), Indonesia (n = 4), and Burundi (n = 1). Some of these strains had been characterized previously for their plasmid profile, DNA fingerprint, and symbiotic ability (8, 10, 12, 19-21). The results shown in Table 1 demonstrate that nodC types α , γ , and δ were represented in the collection, but, unlike the results of isolates from NWA shown in the preceding section, only eight strains showed *nod*C type δ , namely seven strains from Ecuador and one from Peru. *nod*C type α and type γ were detected in 27 (31%) and 57 (61%) strains, respectively. Therefore, if we consider these data together with the above results from isolates from NWA there is a clear difference in the distribution of nodC types in the Americas, where the strains that show type δ are found to cluster in the group of strains from Argentina, Ecuador, and Peru. Indeed, these strains had been isolated from the mid- and southern Andean centers of bean domestication. Conversely, all of the strains from the Mesoamerican pool showed only types α and γ . The same was true of strains from regions other than the Americas (Table 1).

Distribution of nodC Alleles in Rhizobial Populations Retrieved from Latin American Soils. To assess whether the prevalence of each nodC type in the collection of R. etli strains isolated from the different BD centers actually reflects the rhizobial populations present in soils, we examined the isolates retrieved by wild and cultivated common beans from soil samples collected in Mexico, Ecuador, Bolivia, and NWA. A soil suspension from each site was used to cross-inoculate wild common beans representative from the Mesoamerican, the intermediate, and the Andean genetic pools, in all combinations. The Mesoamerican cultivar Negro Xamapa (G3645) was also included in this survey. These bean accessions have been characterized previously by other researchers for phaseolin type, RFLP, and amplified RFLP (1-5, 22, 23). Rhizobia that were isolated from nodules formed in plants from either the Mesoamerican or Andean genetic pools and inoculated with a soil suspension from Mexico showed *nod*C types α and γ (Table 2). In contrast to this, plants inoculated with soil suspensions from Bolivia and from NWA resulted in nodules occupied by mostly *nod*C type δ rhizobia and *nod*C types β and δ rhizobia, respectively (Table 2). Nodules from plants inoculated with the Ecuadorian soil samples showed the four nodC types. The geographic distribution of the nodC alleles among these soils and in the collection of strains examined in the preceding section show a close correlation.

We concluded that the bean rhizobial populations retrieved from Mesoamerican soils by bean plants were clearly different from those retrieved from southern Andes (Bolivia and NWA) soils. Two of the *nod*C alleles, namely β and δ , appear absent only from the Mesoamerican soils. In particular, the population from Ecuador seems to be intermediate; all four alleles so far identified are present. Thus, Ecuador appears as a center of divergence (or convergence) of lineages of the species *R. etli*. Overall, these data indicated the following. (*i*) Bean plant populations of different origins are able to form nodules with rhizobia of each *nod*C type that are found to be predominant in each region, therefore ruling out the possibility that plants from either Mesoamerican or Andean centers restrict nodulation by rhizobia present in soils from other regions. (*ii*) *R. etli* lineages appear to be differently represented in soils from the three major BD centers. (*iii*) The fact that, for each

soil sample, all wild accessions and cultivars were inoculated with the same soil–rhizobial suspension and grown in the same physiological conditions ensured that, in each case, the differences in nodule occupation by the four *R. etli* lineages arose from intrinsic differences in the interactions between both symbionts, and these were more favorable when both had the same geographic origin, suggesting some mutual symbiotic selectivity.

Distribution of Prevalence Among Wild and Cultivated Common Bean Populations from Different Centers of Diversity. To examine the above advanced proposition that in each region the native host plant and R. etli have developed selectivity for each other, we carried out coinoculation experiments with wild and cultivated beans from the three BD centers. Six and 16 wild common bean accessions from the Mesoamerican and Andean pools, respectively, were assayed. In addition, six and two cultivars of Mesoamerican and Andean origin, respectively, were included in this analysis (Table 4, which is published as supporting information on the PNAS web site). Most of these materials had been previously typed for phaseolin (1-5, 22-24). The group of Andean wild accessions comprises representatives of the intermediate and Andean gene pools, respectively. Mixtures of two *R. etli* strains representing the *nod*C types α and δ were used for plant inoculation. *nod*C type α strains CFNX5 (isolated in Mexico) and SC15 (isolated in NWA) and nodC type δ strains 55N1 and 90N3 (both isolated in NWA) were assayed in these experiments.

Table 3 summarizes the survey of 460 and 1,021 isolates each obtained from a bean nodule removed from plants representing the Mesoamerican and the Andean bean genetic pools, respectively, which had been inoculated with a mix of strains SC15 and 55N1. *nod*C type α was by far the dominant pattern in isolates from the Mesoamerican wild and cultivated beans: *nod*C type δ was found in only 3% of nodules (n = 13) formed on six Mesoamerican accessions and six cultivars with Mesoamerican introgressions from Mexico, El Salvador, Guatemala, Colombia, Brazil, and Argentina. In contrast, the average proportion of nodules formed individually by strain *nod*C type δ was comparatively much higher on the 16 wild and 2 cultivated beans from the Andean region (37%). This survey showed also that 100% of nodules sampled from the Mexican accessions G2771 (n = 61) and Negro Xamapa (n = 76) and from the Colombian accessions G24651 (n = 32) and 24683 (n = 37) displayed the *nod*C type α pattern. The strain *nod*C type α was also found occupying nodules formed in beans from the Andean region, albeit at much lower rates. Rates of occupancy by *nod*C type δ were found to be significantly higher than the average in some of the wild accessions from the southern Andes regions, such as G21245 (70%), G10025 (60%), G24318 (50%), G19894 (50%), G23724 (44%), G23445B (43%), G23583 (40%), G23454B (40%), and G19890 (30%). Also, *nod*C allele δ was found to be significantly represented in nodules formed in the Chilean cultivated beans G4474 (23%) and the Argentinean cv. Alubia Cerrillos (65%). Similar results were obtained by coinoculating with mixtures of strains CFNX5 plus 55N1 and SC15 plus 90N3, respectively (data not shown). We concluded that beans from the Mesoamerican and Andean genetic pools were different in their quantitative preference for nodulation by each of the two lineages of *R. etli* examined. Expression of this host-strain interaction was quite strong in the case of the Mesoamerican beans.

Discussion

R. etli is the predominant *P. vulgaris*-nodulating species in the Americas. Our search for intraspecific diversity within the *R. etli* populations found associated to wild common beans from the southern extreme of the Andean BD center led us to identify polymorphism in the *nod*C gene. The use of molecular markers such as phaseolin, isozymes, RFLP, and amplified RFLP have been instrumental in identifying the major Mesoamerican and Andean groups in *P. vulgaris* (4, 5, 24). Similarly, here we

Table 1. *nod*C typing of *R. etli* strains from different geographical origins

Table 1. (continued)

Strain type Source* Strain type Source* American origin - <th></th> <th colspan="2">nodC</th> <th></th> <th>nodC</th> <th colspan="2">nodC</th>		nodC			nodC	nodC	
American origin 1481 y G. Bernal USA Y P. Graham P-1 Y P. Lezama V33 Y P. Graham P-1 Y P. Lezama V33 Y P. Graham Brail Y P. Lezama V36 Y P. Graham Brail Y E. Martinez-Romero Mexico E. Martinez-Romero MH3 Y E. Martinez-Romero F8 Y E. Martinez-Romero MH4 Y M. Hungria F8 Y E. Martinez-Romero MH14 Y M. Hungria F85 Y E. Martinez-Romero MH13 Y M. Hungria F85 Y E. Startinez-Romero MH14 Y M. Hungria F85 Y E. Startinez-Romero MH14 Y M. Hungria F85 Y C. Startize (25) MH37 a M. Hungria F855 Y C. Startize (25) MH43 Y M. Hungria	Strain	type	Source*	Strain	type	Source*	
Detail Peru Peru Second V33 y P. Graham P-1 S. P. Lezana V33 y P. Graham Revit P. Lezana V36 y P. Graham Brazil KinSS (CAT7139) Y K. Losephon Bris Y E. Martinez-Romero Metco Metainez-Romero MH3 Y M. Hungria F6 a E. Martinez-Romero MH16 a M. Hungria GRM2 a E. Martinez-Romero MH13 Y M. Hungria GRM2 a E. Martinez-Romero MH13 Y M. Hungria B1855 a C. Siko (23) MH45 w M. Hungria B1854 a C. Siko (23) MH45 w M. Hungria B1854 a C. Siko (23) GR14 a J. Sanjuan (9) B1854 a C. Siko (23) GR14 a J. Sanjuan (9) B1877 a C. Siko (American origin			1/81	21	G Bernal	
U33 y P. Graham P.4 y P. Learna V36 y P. Graham Brazil P.4 y P. Learna V36 y P. Graham Brazil P.4 y P. Martinez-Romero Tail82 a E. Martinez-Romero MH9 y I. Menyria F6 a E. Martinez-Romero MH9 y I. Menyria F8 y E. Martinez-Romero MH14 y M. Hunyria GYN1 y E. Martinez-Romero MH16 y M. Hunyria BS0 y C. Silva (25) MH33 y M. Hunyria BS1 a C. Silva (25) MH31 y M. Hunyria BS2 y C. Silva (25) MH32 y M. Hunyria BS2 y C. Silva (25) MH32 y M. Hunyria BS3 y C. Silva (25) GR14 a J. Sanjaan (9) BS2 S. Brom (9) GR				Poru	Ŷ	G. Bernar	
V33 / P. Graham P.4 o P. Leasm V36 / P. Graham Bazil P. E. Martinez-Romero Mesilo ////////////////////////////////////	V/23	24	P. Graham	P_1	8	P Lozama	
V33 Y F. Graham Fran Y F. Lezalia Vandis C(LAT7128) Y P. Graham BC Y E. Martinez.Romero Medico E. Martinez.Romero NA Y E. Martinez.Romero Medico E. Martinez.Romero MH3 Y M. Hungria FR E. Martinez.Romero MH3 Y M. Hungria GR1 Y E. Martinez.Romero MH14 Y M. Hungria GR3 E. Martinez.Romero MH13 Y M. Hungria IES51 C. Sitva (25) MH33 Y M. Hungria IES54 Y C. Sitva (25) MH45 G M. Hungria IES54 Y C. Sitva (25) MH45 G M. Hungria IES720 G C. Sitva (25) GR41 G J. Sanjuan (9) IES817 G S. Sinva (25) GR41 G J. Sanjuan (9) IES810 Y S. Sinva (25) GR41 G J. Sanjuan (9)	V23	Ŷ	F. Glallalli	F-I	0		
Visit P - Martham BEAD Kind S (CLAT7139) Y K. Josephon Decembra Y E. Martinez Romero Trill S2 a E. Martinez Romero CRATS12 Y J. Miching It (8) File a E. Martinez Romero MH3 Y M. Hongria File Y E. Martinez-Romero MH14 Y M. Hongria CRN1 Y E. Martinez-Romero MH16 a M. Hongria CRN1 A E. Martinez-Romero MH13 Y M. Hongria LES51 a C. Silva (25) MH33 A M. Hongria LES54 Y C. Silva (25) MH43 Y M. Hongria LE2730 a C. Silva (25) GR14 a J. Sinjuan (9) LE4810 Y C. Silva (25) GR14 a J. Sinjuan (9) LE4810 Y P. Graham Z.1MZ2 a J. Sinjuan (9) LE4810 Y P. Graham Z.1MZ2 <	V35	γ	P. Graham	F-4	γ	P. Lezama	
Rm35 (LA1/139) Y K. Jösephön Bis Y E. Martinez-Komero Metxico Metxico Metxico Metxico Y E. Martinez-Romero MH33 Y M. Hungria F6 a E. Martinez-Romero MH41 Y M. Hungria F8 Y E. Martinez-Romero MH414 Y M. Hungria CPN12 a E. Martinez-Romero MH413 Y M. Hungria CPN2 a E. Martinez-Romero MH413 Y M. Hungria CPN2 a C. Silva (25) MH33 Y M. Hungria LESS1 a C. Silva (25) Other origins Y M. Hungria LESS10 Y C. Silva (25) Spain Seconyan (9) EG33 a J. Sanjuan (9) LESS10 Y P. Silva (25) Spain Seconyan (9) EG33 a J. Sanjuan (9) LESS10 Y P. Silva (25) Spain Seconyan (9) EG33 a J. Sanjua	V30	γ	P. Granam	Brazii			
Tatl2 a E. Martinez-Romero CMPA/S12 y J. Michele (18) Mexico MH3 Y M. Hungria Y M. Hungria F6 a E. Martinez-Romero MH14 Y M. Hungria CN1 Y E. Martinez-Romero MH16 a M. Hungria CN1 Y E. Martinez-Romero MH13 Y M. Hungria B550 Y C. Silva (25) MH33 Y M. Hungria B554 A C. Silva (25) MH33 Y M. Hungria B554 A C. Silva (25) MH45 Y M. Hungria B554 A C. Silva (25) Other origins Hungria Y B557 A C. Silva (25) Other origins Hungria Y Hungria B1827 A Silva (25) Other origins Hungria Y Hungria B1837 Y C. Silva (25) Genta T Sinjjiiii (9) Hungria Y<	KIM55 (CIAT/139)	γ	K. Josephson	Br5	γ	E. Martinez-Romero	
Mekicic MH3 Y M. Hungria F6 a E. Martinez-Romero MH14 Y M. Hungria R8 Y E. Martinez-Romero MH14 Y M. Hungria CPN1 Y E. Martinez-Romero MH13 Y M. Hungria CPN2 a C. Siva (25) MH37 a M. Hungria IES51 a C. Siva (25) MH33 Y M. Hungria IES73 a C. Siva (25) MH48 Y M. Hungria IES73 a C. Siva (25) Other origins M. Hungria IES737 Y C. Siva (25) GR14 a J. Sanjuan (9) IER810 Y C. Siva (25) GR14 a J. Sanjuan (9) UMR130 Y P. Graham SIV12 a J. Sanjuan (9) UMR130 Y P. Graham SIP 27 a J. Sanjuan (9) UMR1311 Y P. Graham SIP 27 a D. Rodriguez-Navaro	Tal182	α	E. Martinez-Romero	CNPAF512	γ	J. Michiels (18)	
F6 a E. Martinez.Romero MH14 y M. Hungria CYN1 Y E. Martinez.Romero MH15 a M. Hungria CYN2 A E. Martinez.Romero MH19 y M. Hungria E950 Y C. Sika (25) MH31 y M. Hungria E951 a C. Sika (25) MH33 y M. Hungria E954 Y C. Sika (25) MH45 a M. Hungria E12730 a C. Sika (25) Other origins M. Hungria (9) E12737 Y C. Sika (25) GR10 Y I. Sanjuan (9) E12810 Y C. Sika (25) GR14 a I. Sanjuan (9) E14804 Y P. Graham S. Foro (9) GG3 a I. Sanjuan (9) UMR1310 Y P. Graham S. Foro (9) GG3 a I. Sanjuan (9) UMR1310 Y P. Graham S. Foro (9) GG3 a I. Sanjuan (9) UMR1313 Y P. Graham S. Foro (9) S. Grom (9) R.	Mexico			MH3	γ	M. Hungría	
F8 y E. Martinez-Romero MH14 y M. Hungria CFN1 A E. Martinez-Romero MH19 y M. Hungria IE950 C. Sliva (25) MH31 y M. Hungria IE954 C. Sliva (25) MH37 a M. Hungria IE954 C. Sliva (25) MH48 y M. Hungria IE2730 C. Sliva (25) MH48 y M. Hungria IE2737 Y C. Sliva (25) GR10 y J. Sanjuan (9) IE4810 Y C. Sliva (25) GR11 y J. Sanjuan (9) IE4817 G C. Sliva (25) GR11 y J. Sanjuan (9) UMR1307 Y P. Graham 21NJ2 a J. Sanjuan (9) UMR1307 Y P. Graham ISP-37 a D. Rodriguez-Navaro (23) UMR1311 Y P. Graham ISP-37 y D. Rodriguez-Navaro (23) UMR1313 Y P. Graham ISP-37 y D. Rodriguez-Navaro (23) UMR1311 Y P. Graham ISP-37	F6	α	E. Martínez-Romero	MH9	α	M. Hungría	
CFN1 7 E. Martinez-Romero MH16 a M. Hungria IE950 7 C. Silva (25) MH31 7 M. Hungria IE951 a C. Silva (25) MH37 a M. Hungria IE954 7 C. Silva (25) MH35 a M. Hungria IE974 a C. Silva (25) MH48 y M. Hungria IE2737 7 C. Silva (25) Other origins M. Hungria M. Hungria IE2737 7 C. Silva (25) Spain J. Sanjuan (9) M. Hungria IE2737 7 C. Silva (25) GR14 a J. Sanjuan (9) IE4830 7 C. Silva (25) GR14 a J. Sanjuan (9) IM1313 7 P. Graham ISP-19 y D. Rodriguez-Navarro (23) UM13130 7 P. Graham ISP-34 a D. Rodriguez-Navarro (23) UM13131 7 P. Graham ISP-37 y D. Rodriguez-Navarro (23) <td< td=""><td>F8</td><td>γ</td><td>E. Martínez-Romero</td><td>MH14</td><td>γ</td><td>M. Hungría</td></td<>	F8	γ	E. Martínez-Romero	MH14	γ	M. Hungría	
CFNA2 a E. Martinez-Romero MH19 y M. Hungria IE950 Y C. Silva (23) MH37 a M. Hungria IE954 Q C. Silva (23) MH37 a M. Hungria IE954 Q C. Silva (23) MH45 a M. Hungria IE2730 Q C. Silva (25) MH46 y M. Hungria IE4840 Y C. Silva (25) GR10 y J. Sanjuan (9) IE4840 Y C. Silva (25) GR14 a J. Sanjuan (9) IE4840 Y C. Silva (25) GR17 a J. Sanjuan (9) IE4840 Y P. Graham SP-19 Y D. Rodriguez Navarro (23) UMR1307 Y P. Graham SP-34 a D. Rodriguez Navarro (23) UMR1311 Y P. Graham SP-34 a D. Rodriguez Navarro (23) UMR1311 Y P. Graham SP-40 Y D. Rodriguez Navarro (23) UMR1313	CFN1	γ	E. Martínez-Romero	MH16	α	M. Hungría	
IB950 γ C. Sliva (25) MH31 γ M. Hungria IB954 γ C. Sliva (25) MH39 γ M. Hungria IB94 γ C. Sliva (25) MH46 γ M. Hungria IB94 α C. Sliva (25) MH46 γ M. Hungria IB2707 γ C. Sliva (25) Gfbra M. Hungria IB2737 γ C. Sliva (25) Gfbra J. Sanjuan (9) IE4840 γ C. Sliva (25) Gfbra J. Sanjuan (9) IE4837 α C. Sliva (25) Gfbra J. Sanjuan (9) GRV α J. Sanjuan (9) Gfbra J. Sanjuan (9) UMR1310 γ P. Graham ISP-27 α D. Rodriguez.Navaro (23) UMR1311 γ P. Graham ISP-37 γ D. Rodriguez.Navaro (23) UMR1311 γ P. Graham ISP-37 γ D. Rodriguez.Navaro (23) UMR1311 γ P. Graham ISP-37 γ D. Rodr	CFN42	α	E. Martínez-Romero	MH19	γ	M. Hungría	
IE951 α C. Silva (25) MH37 α M. Hungria IE954 γ C. Silva (25) MH45 α M. Hungria IE2237 γ C. Silva (25) MH46 α M. Hungria IE2237 γ C. Silva (25) Other origins M. Hungria IE4810 γ C. Silva (25) GR10 γ J. Sanjuan (9) IE4837 α C. Silva (25) GR14 α J. Sanjuan (9) IE4837 α C. Silva (25) GR14 α J. Sanjuan (9) IE4837 α S. Brom (9) BG3 α J. Sanjuan (9) UMN1307 γ P. Graham ISP-19 γ D. Rodriguez-Navarro (23) UMN1313 γ P. Graham ISP-34 α D. Rodriguez-Navarro (23) UMN1313 γ P. Graham ISP-34 α D. Rodriguez-Navarro (23) UMN1313 γ P. Graham ISP-34 α D. Rodriguez-Navarro (23) UMN1313	IE950	γ	C. Silva (25)	MH31	γ	M. Hungría	
IE954 γ C. Silva (25) MH39 γ M. Hungria IE934 a C. Silva (25) MH48 γ M. Hungria IE2230 a C. Silva (25) Other origins J. Sanjuan (9) IE4804 γ C. Silva (25) GR10 γ J. Sanjuan (9) IE4837 a C. Silva (25) GR14 a J. Sanjuan (9) CNNCS a S. Brom (9) GR37 a J. Sanjuan (9) UMR1310 γ P. Graham ISP-27 a D. Rodriguez-Navaro (23) UMR1311 γ P. Graham ISP-37 γ D. Rodriguez-Navaro (23) UMR1313 γ P. Graham ISP-37 γ D. Rodriguez-Navaro (23) UMR1311 γ P. Graham ISP-37 γ D. Rodriguez-Navaro (23) UMR1313 γ P. Graham ISP-41 γ D. Rodriguez-Navaro (23) UMR1314 γ K. Josephon ISP-41 γ D. Rodriguez-Navaro (23) <	IE951	ά	C. Silva (25)	MH37	ά	α M. Hungría	
iso c Silva (25) MH45 a M. Hungria IE2730 a C. Silva (25) Other origins Use IE2737 Y C. Silva (25) Spain Use IE4804 Y C. Silva (25) GR10 Y J. Sanjuan (9) IE4817 a C. Silva (25) GR14 a J. Sanjuan (9) IE4817 a C. Silva (25) GR14 a J. Sanjuan (9) IE4817 a C. Silva (25) GR17 a J. Sanjuan (9) UMR1310 Y P. Graham ISP-19 Y D. Rodriguez-Navaro (23) UMR1313 Y P. Graham ISP-37 Y D. Rodriguez-Navaro (23) UMR1313 Y P. Graham ISP-40 Y D. Rodriguez-Navaro (23) ILPMx112 Y This laboratory ISP-41 Y D. Rodriguez-Navaro (23) ILPMx113 Y K. Josephan ISP-44 Y D. Rodriguez-Navaro (23) ILPMx112 Y	IE954	2	C Silva (25)	MH39	γ	v M. Hungría	
IE2730 a C. Silva (25) MH48 y M. Hungris IE2737 Y C. Silva (25) Spain	IF994	a a	C Silva (25)	MH45	α α	M Hungría	
IE2337 Q C. Silva (25) Other origins I. Knight IE24804 Q C. Silva (25) GR10 Y J. Sanjuan (9) IE4810 Q C. Silva (25) GR14 a J. Sanjuan (9) IE4817 a C. Silva (25) GR14 a J. Sanjuan (9) VMR1307 Y P. Graham ISP-19 Y D. Rodriguez-Navarro (23) UMR1310 Y P. Graham ISP-19 Y D. Rodriguez-Navarro (23) UMR1313 Y P. Graham ISP-37 Y D. Rodriguez-Navarro (23) UMR1313 Y P. Graham ISP-37 Y D. Rodriguez-Navarro (23) UMR1313 Y P. Graham ISP-40 Y D. Rodriguez-Navarro (23) UMR1313 Y E. Martinez-Romero ISP-42 a D. Rodriguez-Navarro (23) Olivia 4 Y E. Martinez-Romero ISP-42 a D. Rodriguez-Navarro (23) Olivia 4 Y S. Brom (9) ISP-35 Y D. Ro	152730	a	$C_{\rm Silva}(25)$	MH/8			
IE404 y C. Silva (25) Spain IE404 y C. Silva (25) GR10 y J. Sanjuan (9) IE4837 a C. Silva (25) GR14 a J. Sanjuan (9) CFNx5 a S. Brom (9) BG87 a J. Sanjuan (9) UMR1307 y P. Graham SIP19 y D. Rodriguez-Navarro (23) UMR1311 y P. Graham ISP-19 y D. Rodriguez-Navarro (23) UMR1313 y P. Graham ISP-37 a D. Rodriguez-Navarro (23) UMR1313 y P. Graham ISP-37 y D. Rodriguez-Navarro (23) UMR1313 y P. Graham ISP-37 y D. Rodriguez-Navarro (23) UPMx112 y This laboratory ISP-37 y D. Rodriguez-Navarro (23) Olivia 4 y E. Martinez-Romero ISP-42 a D. Rodriguez-Navarro (23) Olivia 4 y E. Martinez-Romero ISP-43 y D. Rodriguez-Navarro (23) ClAT896 y CLAT894 y S. Brom (9) ISP-35	122730	a	$C_{\rm Silva}(25)$	Other origins	Ŷ	y w. nuligria	
IE4800 IE4810YC. Silva (25)SpainIE4810YC. Silva (25)GR10YJ. Sanjuan (9)IE4837 α C. Silva (25)GR14 α J. Sanjuan (9)IE4837 α C. Silva (25)GR14 α J. Sanjuan (9)CFNX5 α S. Brom (9)BC3 α J. Sanjuan (9)UMR1307YP. GrahamS. P. 27 α D. Rodriguez-Navarro (23)UMR1311YP. GrahamISP-27 α D. Rodriguez-Navarro (23)UMR1313YP. GrahamISP-34 α D. Rodriguez-Navarro (23)LPMx101YThis laboratoryISP-40YD. Rodriguez-Navarro (23)UMR1313YP. GrahamISP-41YD. Rodriguez-Navarro (23)UMR1314YK. JosephsonISP-42 α D. Rodriguez-Navarro (23)Olivia 4YE. Martinez-RomeroISP-42 γ D. Rodriguez-Navarro (23)Olivia 4YS. Brom (9)ISP-35YD. Rodriguez-Navarro (23)CIAT894YS. Brom (9)ISP-35YD. Rodriguez-Navarro (23)CIAT903 α CIATISP-64YD. Rodriguez-Navarro (23)CIAT904YCIATISP-64YD. Rodriguez-Navarro (23)CIAT905 α CIATISP-64YD. Rodriguez-Navarro (23)CIAT904YP. GrahamGD131YN. Amarger1373 δ P. GrahamGD144Y	122737	Ŷ	C. Silva (25)	Statin			
IE4810yC. Silva (25)GR10yI. Sanjuan (9)IE4837aC. Silva (25)GR14aI. Sanjuan (9)CFNX5aS. Brom (9)6R87aI. Sanjuan (9)UMR1307yP. Graham21NU2aI. Sanjuan (9)UMR1310yP. Graham1SP-19yD. Rodriguez-Navarro (23)UMR1311yP. GrahamISP-27aD. Rodriguez-Navarro (23)UMR1313yP. GrahamISP-37yD. Rodriguez-Navarro (23)UMR1313yP. GrahamISP-37yD. Rodriguez-Navarro (23)UPMx112yThis laboratoryISP-40yD. Rodriguez-Navarro (23)BelizeISP-41yD. Rodriguez-Navarro (23)Viking 1yK. JosephsonColombiaISP-44yD. Rodriguez-Navarro (23)Viking 1yR. Rodriguez-Navarro (23)CiAT895yCIATISP-38yD. Rodriguez-Navarro (23)CiAT903aCIATISP-64yD. Rodriguez-Navarro (23)CiAT7004yCIATISP-64yD. Rodriguez-Navarro (23)CiAT704aP. GrahamGD261yN. Amarger1373 δ P. GrahamGD261yN. Amarger1374aP. GrahamGD261yN. Amarger1374 δ P. GrahamGD261yN. Amarger1373 δ P. GrahamGD261yN. Amarger	154004	γ		Spain			
IE4837aC. Silva (25)GR14aI. Sanjuan (9)CFNX5aS. Brom (9)GR87aI. Sanjuan (9)UMR1307YP. Graham21NU2aI. Sanjuan (9)UMR1310YP. GrahamISP-19YD. Rodriguez-Navarro (23)UMR1311YP. GrahamISP-27aD. Rodriguez-Navarro (23)UMR1313YP. GrahamISP-34aD. Rodriguez-Navarro (23)LPMX101YThis laboratoryISP-34aD. Rodriguez-Navarro (23)LPMX112YThis laboratoryISP-40YD. Rodriguez-Navarro (23)Olivia 4YE. Martinez-RomeroISP-42aD. Rodriguez-Navarro (23)Olivia 4YE. Martinez-RomeroISP-42aD. Rodriguez-Navarro (23)Viking 1YK. JosephsonISP-35YD. Rodriguez-Navarro (23)ColombiaISP-35YD. Rodriguez-Navarro (23)C(AT895YD. Rodriguez-Navarro (23)C(AT903aCIATISP-52aD. Rodriguez-Navarro (23)C(AT7064YCIATISP-64YD. Rodriguez-Navarro (23)C(AT7074QCIATISP-64YD. Rodriguez-Navarro (23)C(AT7064YP. GrahamGD164YN. Amarger1374aP. GrahamGD261YN. Amarger1374aP. GrahamGD261YN. Amarger1374aP. GrahamGD26	IE4810	γ	C. SIIva (25)	GR10	γ	J. Sanjuan (9)	
CFNX5 a S. Brom (9)GR87 a I. Sanjuan (9)UMR1307 γ P. Graham21NU2 a I. Sanjuan (9)UMR1310 γ P. Graham15P-19 γ D. Rodriguez-Navarro (23)UMR1311 γ P. GrahamISP-27 a D. Rodriguez-Navarro (23)UMR1313 γ P. GrahamISP-37 γ D. Rodriguez-Navarro (23)LPMX.101 γ This laboratoryISP-37 γ D. Rodriguez-Navarro (23)LPMX.112 γ This laboratoryISP-37 γ D. Rodriguez-Navarro (23)BelizeISP-41 γ D. Rodriguez-Navarro (23)Olivia 4 γ E. Martinez-RomeroISP-42 a D. Rodriguez-Navarro (23)ClartsesISP-45 γ D. Rodriguez-Navarro (23)Clartses γ S. Brom (9)ISP-35 γ D. Rodriguez-Navarro (23)Clartses γ G. Rodriguez-Navarro (23)Clartses γ D. Rodriguez-Navarro (23)C	IE4837	α	C. Silva (25)	GR14	α	J. Sanjuan (9)	
FN-11yS. Brom (9)8C3 α J. Sanjuan (9)UMR1307yP. GrahamISP-19yD. Rodriguez-Navarro (23)UMR1310yP. GrahamISP-27 α D. Rodriguez-Navarro (23)UMR1313yP. GrahamISP-34 α D. Rodriguez-Navarro (23)LPMX101yThis laboratoryISP-34 α D. Rodriguez-Navarro (23)LPMX112yThis laboratoryISP-40yD. Rodriguez-Navarro (23)BelizeISP-41yD. Rodriguez-Navarro (23)Rodriguez-Navarro (23)Olivia 4yE. Martinez-RomeroISP-44yD. Rodriguez-Navarro (23)Olivia 4yE. Martinez-RomeroISP-44yD. Rodriguez-Navarro (23)ClAT894yS. Brom (9)ISP-35yD. Rodriguez-Navarro (23)ClAT894yS. Brom (9)ISP-35yD. Rodriguez-Navarro (23)ClAT903 α CIATISP-60yD. Rodriguez-Navarro (23)ClAT7004yCIATISP-68yD. Rodriguez-Navarro (23)ClAT7014 α CIATISP-68yD. Rodriguez-Navarro (23)1640yP. GrahamGD261yN. Amarger1374 α P. GrahamGD261yN. Amarger1374 α P. GrahamGD261yN. Amarger1374 α P. GrahamRecherche Agronomique, Dijon, France, K. Josephson, Department of Soli, Refraham (8)Burundi <td>CFNX5</td> <td>α</td> <td>S. Brom (9)</td> <td>GR87</td> <td>α</td> <td colspan="2">J. Sanjuan (9)</td>	CFNX5	α	S. Brom (9)	GR87	α	J. Sanjuan (9)	
UMR1307yP.Graham21N2aJ. Sanjuan (9)UMR1310yP. GrahamISP-19yD. Rodriguez-Navarro (23)UMR1311yP. GrahamISP-27aD. Rodriguez-Navarro (23)UMR1313yP. GrahamISP-37yD. Rodriguez-Navarro (23)LPMx110yThis laboratoryISP-40yD. Rodriguez-Navarro (23)BelizeISP-41yD. Rodriguez-Navarro (23)Olivia 4yE. Martinez-RomeroISP-42aD. Rodriguez-Navarro (23)ColombiaISP-45yD. Rodriguez-Navarro (23)ColombiaISP-45yD. Rodriguez-Navarro (23)ColarbaiISP-45yD. Rodriguez-Navarro (23)ColarbaiISP-45yD. Rodriguez-Navarro (23)ColarbaiISP-45yD. Rodriguez-Navarro (23)ColarbaiISP-45yD. Rodriguez-Navarro (23)ColarbaiG. CATISP-52aD. Rodriguez-Navarro (23)ColarbaiaCIATISP-64yD. Rodriguez-Navarro (23)ColarbaiaCIATISP-68yD. Rodriguez-Navarro (23)ColarbaiaCIATISP-69yD. Rodriguez-Navarro (23)ColarbaiaP. GrahamGD161yN. Amarger1376AP. GrahamGD161yN. Amarger1377SP. Graham (8)CP91yN. Amarger1378yP. Graham (8) <t< td=""><td>FNJ-11</td><td>γ</td><td>S. Brom (9)</td><td>8C3</td><td>α</td><td colspan="2">J. Sanjuan (9)</td></t<>	FNJ-11	γ	S. Brom (9)	8C3	α	J. Sanjuan (9)	
UMR1310yPGrahamISP-19yD. Rodriguez-Navarro (23)UMR1311yP. GrahamISP-27 α D. Rodriguez-Navarro (23)UMR1313yP. GrahamISP-34 α D. Rodriguez-Navarro (23)LPMA101yThis laboratoryISP-37yD. Rodriguez-Navarro (23)LPMX112yThis laboratoryISP-40yD. Rodriguez-Navarro (23)BelizeISP-41yD. Rodriguez-Navarro (23)Olivia 4yE. Martinez-RomeroISP-42 α D. Rodriguez-Navarro (23)Viking 1yK. JosephsonISP-44yD. Rodriguez-Navarro (23)ColombiaISP-43yD. Rodriguez-Navarro (23)C(AT894yS. Brom (9)ISP-35yD. Rodriguez-Navarro (23)C(AT894yS. Brom (9)ISP-38yD. Rodriguez-Navarro (23)C(AT7004yC(ATISP-83yD. Rodriguez-Navarro (23)C(AT904yC(ATISP-60yD. Rodriguez-Navarro (23)C(AT7004qC(ATISP-69yD. Rodriguez-Navarro (23)C(AT7014 α C(ATISP-69yD. Rodriguez-Navarro (23)Io02yP. GrahamIndonesiaEcuadorIndonesiaIndonesiaIndonesiaIndonesiaIndonesiaIndonesiaIndonesia1376 β P. Graham (8)CP91yN. AmargerI383yP. GrahamRecherche Agronomique, Djion, France; K. Josephson, Department o	UMR1307	γ	P. Graham	21NJ2	α	J. Sanjuan (9)	
UMR1311γP. GrahamISP-27αD. Rodriguez-Navarro (23)UMR1313γP. GrahamISP-34αD. Rodriguez-Navarro (23)LPMx101γThis laboratoryISP-37γD. Rodriguez-Navarro (23)LPMx112γThis laboratoryISP-40γD. Rodriguez-Navarro (23)BelizeISP-41γD. Rodriguez-Navarro (23)Olivia 4γE. Martinez-RomeroISP-42αD. Rodriguez-Navarro (23)ColombiaISP-45γD. Rodriguez-Navarro (23)ColombiaISP-45γD. Rodriguez-Navarro (23)ClAT995γCIATISP-35γD. Rodriguez-Navarro (23)ClAT903αCIATISP-52αD. Rodriguez-Navarro (23)ClAT903αCIATISP-60γD. Rodriguez-Navarro (23)ClAT704γCIATISP-64γD. Rodriguez-Navarro (23)ClAT705αCIATISP-64γD. Rodriguez-Navarro (23)ClAT706αCIATISP-68γD. Rodriguez-Navarro (23)1002γP. GrahamIndonesiaTEcuadorGD131γN. Amarger1374αP. GrahamGD261γN. Amarger1375δP. Graham (8)BC15αN. Amarger1376γP. Graham (8)BC15αN. Amarger1376βG. Garbam (8)CP11γN. Amarger1378γP.	UMR1310	γ	P. Graham	ISP-19	γ	D. Rodriguez-Navarro (23)	
UMR 1313yP. GrahamISP-34 α D. Rodriguez-Navarro (23)LPMx101yThis laboratoryISP-37yD. Rodriguez-Navarro (23)LPMx112yThis laboratoryISP-40yD. Rodriguez-Navarro (23)BelizeISP-41yD. Rodriguez-Navarro (23)Olivia 4yE. Martinez-RomeroISP-44yD. Rodriguez-Navarro (23)ColombiaISP-44yD. Rodriguez-Navarro (23)ClatsesColombiaISP-35yD. Rodriguez-Navarro (23)ClAT894yS. Brom (9)ISP-35yD. Rodriguez-Navarro (23)ClAT895yClATISP-38yD. Rodriguez-Navarro (23)ClAT903 α CLATISP-60yD. Rodriguez-Navarro (23)ClAT7004yCLATISP-64yD. Rodriguez-Navarro (23)ClAT7014 α CLATISP-68yD. Rodriguez-Navarro (23)ClAT7014 α CLATISP-68yD. Rodriguez-Navarro (23)I640yP. GrahamIndonesiaIndonesiaIndonesiaEcuadorGD151yN. Amarger1373AP. Graham (B)BurundiIndonesia1376AP. Graham (8)BurundiIndonesia1377SP. Graham (8)BurundiIndonesia1385yP. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil, EcAA383YP. GrahamRecherche Agronomi	UMR1311	γ	P. Graham	ISP-27	α	D. Rodriguez-Navarro (23)	
LPMx101yThis laboratoryISP-37yD. Rodriguez-Navarro (23)LPMx112yThis laboratoryISP-40yD. Rodriguez-Navarro (23)BelizeISP-41yD. Rodriguez-Navarro (23)Olivia 4yE. Martinez-RomeroISP-42 α D. Rodriguez-Navarro (23)ColombiayK. JosephsonISP-44yD. Rodriguez-Navarro (23)ColombiaISP-45yD. Rodriguez-Navarro (23)ClAT895yClATISP-35yD. Rodriguez-Navarro (23)ClAT895yClATISP-35yD. Rodriguez-Navarro (23)ClAT903 α ClATISP-52 α D. Rodriguez-Navarro (23)ClAT704 γ ClATISP-64yD. Rodriguez-Navarro (23)ClAT705 α CLATISP-68yD. Rodriguez-Navarro (23)ClAT7106 α CLATISP-68yD. Rodriguez-Navarro (23)1640yP. GrahamIndonesiaTTEcuadorGD131yN. Amarger1376 δ P. Graham (8)BurundiT1377 δ P. Graham (8)BurundiT1383yP. Graham (8)Burundi*N. Amarger1385yP. GrahamRecherche Agronomique, Dijon, France, K. Josephson, Department of Soil,1385yG. GernalMarger, Laboratorie de Microbiologie des Sols, Institut Nacional de la1386gG. GernalRecherche Agronomique,	UMR1313	γ	P. Graham	ISP-34	α	D. Rodriguez-Navarro (23)	
LPMx112γThis laboratoryISP-40γD. Rodriguez-Navarro (23)BelizeISP-41γD. Rodriguez-Navarro (23)Olivia 4γE. Martinez-RomeroISP-42αD. Rodriguez-Navarro (23)Viking 1γK. JosephsonISP-44γD. Rodriguez-Navarro (23)ColombiaISP-45γD. Rodriguez-Navarro (23)ClAT894γS. Brom (9)ISP-35γD. Rodriguez-Navarro (23)ClAT895γC.IATISP-38γD. Rodriguez-Navarro (23)ClAT7004γC.IATISP-64γD. Rodriguez-Navarro (23)ClAT7014αC.IATISP-64γD. Rodriguez-Navarro (23)CLAT7014αC.IATISP-64γD. Rodriguez-Navarro (23)Ib40γP. GrahamISP-69γD. Rodriguez-Navarro (23)1b60αP. GrahamISP-69γD. Rodriguez-Navarro (23)1b73δP. GrahamGD164γN. Amarger1373δP. Graham (8)CP91γN. Amarger1374αP. Graham (8)BurundiN. Amarger1375βP. Graham (8)BurundiN. Amarger1376βG. Graham (8)BurundiN. Amarger1377βP. GrahamRecherche Agronomique, Dijon, France, K. Josephson, Department of Soil, EcAA1383γP. GrahamN. Amarger, Laboratorie de Microbiologie des Sols, Institut Nacional de Ia1376G	LPMx101	γ	This laboratory	ISP-37	γ	D. Rodriguez-Navarro (23)	
BelizeISP-41 γ D. Rodriguez-Navarro (23)Olivia 4 γ E. Martinez-RomeroISP-42 α D. Rodriguez-Navarro (23)Viking 1 γ K. JosephsonISP-44 γ D. Rodriguez-Navarro (23)ColombiaISP-45 γ D. Rodriguez-Navarro (23)ClAT894 γ S. Brom (9)ISP-35 γ D. Rodriguez-Navarro (23)ClAT895 γ ClATISP-38 γ D. Rodriguez-Navarro (23)ClAT903 α ClATISP-52 α D. Rodriguez-Navarro (23)ClAT7004 γ ClATISP-60 γ D. Rodriguez-Navarro (23)ClAT7014 α ClATISP-68 γ D. Rodriguez-Navarro (23)ClAT7106 α ClATISP-68 γ D. Rodriguez-Navarro (23)1002 γ P. GrahamISP-68 γ D. Rodriguez-Navarro (23)1640 γ P. GrahamIndonesiaIndonesiaEcuadorGD131 γ N. Amarger1373 δ P. Graham (8)EUrundi1376 δ P. Graham (8)BUrundi1377 δ P. Graham (8)BC15 α 1383 γ P. Graham (8)BC15 α 1383 γ P. Graham (8)BC15 α 1383 γ P. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soli, Rizohan (8)1376 δ G. BernalRecherche Agronomique, Dijon, Grance; K. Josephson, Department of Soli, Recharche	LPMx112	γ	This laboratory	ISP-40	γ	D. Rodriguez-Navarro (23)	
Olivia 4 γ E. Martinez-RomeroISP-42 α D. Rodriguez-Navarro (23)Viking 1 γ K. JosephsonISP-44 γ D. Rodriguez-Navarro (23)ColombiaISP-45 γ D. Rodriguez-Navarro (23)CIAT894 γ S. Brom (9)ISP-35 γ D. Rodriguez-Navarro (23)CIAT895 γ CIATISP-38 γ D. Rodriguez-Navarro (23)CIAT903 α CIATISP-52 α D. Rodriguez-Navarro (23)CIAT7004 γ CIATISP-64 γ D. Rodriguez-Navarro (23)CIAT7014 α CIATISP-68 γ D. Rodriguez-Navarro (23)IO02 γ P. GrahamISP-69 γ D. Rodriguez-Navarro (23)1002 γ P. GrahamIndonesiaIndonesiaEcuadorGD131 γ N. Amarger1373 δ P. GrahamGD164 γ N. Amarger1376 δ P. Graham (8)CP91 γ N. Amarger1376 δ P. Graham (8)CP91 γ N. Amarger1378 γ P. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,1383 γ P. GrahamRecherche Agronomique, Diion, France; K. Josephson, Department of Soil,Ec4A δ G. BernalWater and Environmental Science, University of Arizona, Tucson; P. Graham,Ec4A δ G. BernalWater And Environmental Science, University of Arizona, Tucson; P. Graham,Ec4A δ G. Be	Belize	,	2	ISP-41	γ	D. Rodriguez-Navarro (23)	
Viking 1 γ K. JosephsonISP-44 γ D. Rodriguez-Navarro (23)ColombiaISP-45 γ D. Rodriguez-Navarro (23)CIAT895 γ CIATISP-35 γ D. Rodriguez-Navarro (23)CIAT895 γ CIATISP-38 γ D. Rodriguez-Navarro (23)CIAT903 α CIATISP-52 α D. Rodriguez-Navarro (23)CIAT7014 α CIATISP-64 γ D. Rodriguez-Navarro (23)CIAT7014 α CIATISP-64 γ D. Rodriguez-Navarro (23)CIAT7014 α CIATISP-64 γ D. Rodriguez-Navarro (23)I002 γ P. GrahamIndonesia β P. GrahamIndonesiaEcuador α P. GrahamGD131 γ N. Amarger1373 δ P. Graham (8)CP91 γ N. Amarger1376 δ P. Graham (8)Burundi α N. Amarger1378 γ P. Graham (8)BC15 α N. Amarger1383 γ P. Graham (8)BC15 α N. Amarger1383 γ G. BernalRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,Ec101 γ G. BernalKater and Environmental Science, University of Arizona, Tucson; P. Graham,Ec20A δ G. Bernalthizebiur Research Laboratory, University of Arizona, Tucson; P. Graham,Ec20A δ G. Bernalthizebiur Research Laboratori of eNitrogeno, UNAM, Cuernavaca, Mex-E	Olivia 4	γ	E. Martinez-Romero	ISP-42	ά	D. Rodriguez-Navarro (23)	
IntroductIs P-45 γ D. Rodriguez-Navarro (23)ClAT894 γ S. Brom (9)ISP-35 γ D. Rodriguez-Navarro (23)CLAT895 γ CLATISP-38 γ D. Rodriguez-Navarro (23)CLAT903 α CLATISP-52 α D. Rodriguez-Navarro (23)CLAT7004 γ CLATISP-60 γ D. Rodriguez-Navarro (23)CLAT7014 α CLATISP-64 γ D. Rodriguez-Navarro (23)CLAT7016 α CLATISP-64 γ D. Rodriguez-Navarro (23)1002 γ P. GrahamISP-69 γ D. Rodriguez-Navarro (23)1040 γ P. GrahamIndonesia z EcuadorGD131 γ N. Amarger1373 δ P. GrahamGD261 γ N. Amarger1376 δ P. Graham (8)Burundi z 1377 δ P. Graham (8)BC15 α N. Amarger1383 γ P. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,1383 γ G. BernalRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,Ec101 γ G. BernalKater and Environmental Science, University of Arizona, Tucson; P. Graham,Ec20A δ G. Bernalicaz G. Bernalicaz G. BernalIcada β G. Bernalicaz G. G. Bernal, Instituto Nacional Autónomo de Inversigaciones Agropecuar-Icada β G. Bernalicaz G. G. Bernal, Instituto Naciona	Viking 1	2	K losephson	ISP-44	v	D Rodriguez-Navarro (23)	
CIANDAγS. Brom (9)ISP-35γD. Rodriguez-Navarro (23)CIAT895γCIATISP-38γD. Rodriguez-Navarro (23)CIAT903αCIATISP-52αD. Rodriguez-Navarro (23)CIAT7004γCIATISP-60γD. Rodriguez-Navarro (23)CIAT7014αCIATISP-64γD. Rodriguez-Navarro (23)CIAT7016αCIATISP-64γD. Rodriguez-Navarro (23)1002γP. GrahamISP-69γD. Rodriguez-Navarro (23)1640γP. GrahamIndonesiaEcuadorGD131γN. Amarger1374αP. GrahamGD261γN. Amarger1376δP. Graham (8)CP91γN. Amarger1378γP. Graham (8)Burundi1383γP. GrahamBC15αN. Amarger1385γP. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,Ec0AδG. BernalRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,Ec101γG. BernalRhizobium Research Laboratory, University of Arizona, Tucson; P. Graham,Ec20AδG. BernalKhizobium Research Laboratory, University of Arizona, Tucson; P. Graham,Ec21γG. BernalCentro Pesquisa Soja, Embrapa, Londrina, Brazil; P. Lezama, Universida1468αP. Graham (8)Centro Pesquisa Soja, Embrapa, Londrina, Brazil; P. Lezam	Colombia	/	itt sosepilisen	ISP-45	7	D. Rodriguez-Navarro (23)	
CIATESTyC. Don'(y)B. 3.2yD. Rodriguez-Navarro (23)CIATSS γ CIATISP-38 γ D. Rodriguez-Navarro (23)CIAT7014 α CIATISP-60 γ D. Rodriguez-Navarro (23)CIAT7014 α CIATISP-64 γ D. Rodriguez-Navarro (23)CIAT7016 α CIATISP-68 γ D. Rodriguez-Navarro (23)1002 γ P. GrahamIsP-69 γ D. Rodriguez-Navarro (23)1040 γ P. GrahamIndonesiaEcuadorEcuador1373 δ P. GrahamGD164 γ N. Amarger1376 δ P. Graham (8)CP91 γ N. Amarger1377 δ P. Graham (8)BurundiT1378 γ P. GrahamRcherche Agronomique, Dijon, France; K. Josephson, Department of Soil,1383 γ P. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,EC4A δ G. BernalRhizobium Research Laboratory, University of Minnesota, St. Paul; E. MarcelEC20A δ G. BernalInitez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex-EC21 γ G. Bernalias, Statiut Nacional de AgrieroHA88 α P. Graham (8)Centro Pesquisa Soja, Embrapa, Londrina, Brazil; P. Lezama, UniversidadHA78 α P. Graham (8)Centro Pesquisa Soja, Embrapa, Londrina, Brazil; P. Lezama, Universidad	CIAT894	24	S Brom (9)	ISP-35	7	D. Rodriguez-Navarro (23)	
CIAT993 γ CIATID-SD γ D. Rodriguez-Navarro (23)CIAT903 α CIATISP-52 α D. Rodriguez-Navarro (23)CIAT7014 α CIATISP-64 γ D. Rodriguez-Navarro (23)CIAT7106 α CIATISP-64 γ D. Rodriguez-Navarro (23)1002 γ P. GrahamISP-69 γ D. Rodriguez-Navarro (23)1640 γ P. GrahamIndonesiaEcuadorGD131 γ N. Amarger1373 δ P. GrahamGD261 γ N. Amarger1374 α P. Graham (8)CP91 γ N. Amarger1377 δ P. Graham (8)BurundiT1378 γ P. Graham (8)Burundi1383 γ P. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil, Eceak1385 γ G. BernalWater and Environmental Science, University of Arizona, Tucson; P. Graham, Rhizobium Research Laboratory, University of Minnesota, St. Paul; E. Mar- Ec20A δ G. BernalEc20A δ G. BernalKatra and Environmental Science, University of Minnesota, St. Paul; E. Mar- ticez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex- ico; G. BernalG. BernalEc20A δ G. Bernalias, Estación Experimental Santa Catalina, Quito, Ecuador; M. Hungria, Centro Pesquisa Soja, Embrapa, Londrina, Brazil; P. Lezama, UniversidadH48 α P. Graham (8)Centro Pesquisa Soja, Embrapa, Londrina, Brazil; P. Lezama, Universidad <td>CIATROF</td> <td>Ŷ</td> <td>CIAT</td> <td>ISD_38</td> <td>7</td> <td>D. Rodriguez-Navarro (23)</td>	CIATROF	Ŷ	CIAT	ISD_38	7	D. Rodriguez-Navarro (23)	
CIAT303 α CIATISP-60 γ D. Rodriguez-Navarto (23)CIAT7014 α CIATISP-60 γ D. Rodriguez-Navarto (23)CIAT7106 α CIATISP-64 γ D. Rodriguez-Navarto (23)1002 γ P. GrahamISP-69 γ D. Rodriguez-Navarto (23)1004 γ P. GrahamIndonesiaEcuadorGD131 γ N. Amarger1373 δ P. GrahamGD164 γ N. Amarger1374 α P. Graham (8)CP91 γ N. Amarger1375 δ P. Graham (8)Burundi α N. Amarger1376 δ P. Graham (8)Burundi α N. Amarger1383 γ P. GrahamBC15 α N. Amarger1383 γ P. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,Ec4A δ G. BernalWater and Environmental Science, University of Arizona, Tucson; P. Graham,Ec4A δ G. BernalWater and Environmental Science, University of Minnesota, St. Paul; E. Martinez/Duite Asset (2004)Ec101 γ G. Bernaltinez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex-Ec20A δ G. Bernaltinez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex-Ec21 γ G. Bernaltinez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex-Ec24A δ G. Bernaltinez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex	CIAT003	Ŷ	CIAT	ISP_52	Ŷ	D. Rodriguez-Navarro (23)	
CIAT/004 γ CIATIsP-60 γ D. Rodriguez-Navarro (23)CIAT7014 α CIATISP-64 γ D. Rodriguez-Navarro (23)1002 γ P. GrahamISP-68 γ D. Rodriguez-Navarro (23)1002 γ P. GrahamIndonesiaEcuadorGD131 γ N. Amarger1373 δ P. GrahamGD164 γ N. Amarger1376 δ P. Graham (8)CP91 γ N. Amarger1377 δ P. Graham (8)BurundiT1378 γ P. Graham (8)BC15 α N. Amarger1383 γ P. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,EctAA1385 γ P. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,EctOl γ G. BernalRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,EctOl γ G. BernalKaizobium Research Laboratory, University of Arizona, Tucson; P. Grahan,EctOl γ G. BernalKizobium Research Laboratory, University of Minnesota, St. Paul; E. Mar-Ec20A δ G. Bernaltinez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex-Ec21 γ G. Bernaltisz Estación Experimental Santa Catalina, Quito, Ecuador; M. Hungria,1468 α P. Graham (8)Critivaz Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri-1478 α P. Graham (8)Privada Antenor Orrego, Trujillo, Perú; C	CIATZOOA	α	CIAT	151-52	u	D. Rodriguez Neverre (22)	
CIAT/014 α CIATIsP-64 γ D. Rodriguez-Navaro (23)CIAT/106 α CIATISP-68 γ D. Rodriguez-Navaro (23)1002 γ P. GrahamIsP-69 γ D. Rodriguez-Navaro (23)1640 γ P. GrahamIndonesiaEcuadorGD131 γ N. Amarger1373 δ P. GrahamGD164 γ N. Amarger1374 α P. GrahamGD261 γ N. Amarger1376 δ P. Graham (8)CP91 γ N. Amarger1378 γ P. Graham (8)Burundi γ N. Amarger1383 γ P. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,1385 γ G. BernalWater and Environmental Science, University of Arizona, Tucson; P. Graham,Ec0A δ G. BernalKhizobium Research Laboratory, University of Minnesota, St. Paul; E. Mar-Ec2A δ G. Bernalicoz, G. Bernal, Institut Nacional Autónomo de Investigaciones Agropecuar-Ec2L γ G. Bernalicoz, G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-Ec2L γ G. Bernalicoz, G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-Ec2AA δ G. Bernalicoz, G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-Ic2AA δ G. Bernalicoz, G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-Ic2AA δ G. Bernalicoz, G. Bernal, Instituto Nacio	CIAT7004	γ	CIAT	ISP-00	γ	D. Rodriguez-Navarro (23)	
CLAT/106 α CLATISP-68 γ D. Rodriguez-Navarro (23)1002 γ P. GrahamISP-69 γ D. Rodriguez-Navarro (23)1640 γ P. GrahamIndonesiaEcuadorGD131 γ N. Amarger1373 δ P. GrahamGD164 γ N. Amarger1374 α P. Graham (8)CP91 γ N. Amarger1376 δ P. Graham (8)BurundiT1378 γ P. Graham (8)BC15 α N. Amarger1383 γ P. GrahamBC15 α N. Amarger1385 γ P. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil, Water and Environmental Science, University of Arizona, Tucson; P. Graham, Ec101 γ G. BernalEc20A δ G. BernalKhizobium Research Laboratory, University of Minnesota, St. Paul; E. Mar- tinez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex- tinez-Romero, Centro sobre Fijación de Nitróge	CIA17014	α	CIAT	ISP-64	γ	D. Rodriguez-Navarro (23)	
1002γP. GrahamISP-69γD. Rodriguez-Navarro (23)1640γP. GrahamIndonesiaEcuadorGD131γN. Amarger1373δP. GrahamGD164γN. Amarger1374αP. Graham (8)CP91γN. Amarger1376δP. Graham (8)BurundiT1377δP. Graham (8)BurundiT1378γP. Graham (8)BC15αN. Amarger1383γP. GrahamRcherche Agronomique, Dijon, France; K. Josephson, Department of Soil,Soil, Institut Nacional de la1385γP. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,Water and Environmental Science, University of Arizona, Tuccon; P. Graham,Ec101γG. Bernaltínez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex-Ec21γG. Bernaltínez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex-Ec24AδG. Bernalico; G. Bernal, Institut Nacional Autónomo de Investigaciones Agropecuar-Ec24AδG. Bernalico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-Ico; G. Bernalico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-Ico; G. Bernalico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-Ico; G. Bernalico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-Ico; G. Bernalico; G. Bernal, Instituto Nacional Autónomo de Investigacione	CIAT7106	α	CIAT	ISP-68	γ	D. Rodriguez-Navarro (23)	
1640 γ P. GrahamIndonesiaEcuadorGD131 γ N. Amarger1373 δ P. GrahamGD164 γ N. Amarger1374 α P. GrahamGD261 γ N. Amarger1376 δ P. Graham (8)CP91 γ N. Amarger1377 δ P. Graham (8)Burundi1378 γ P. GrahamBC15 α N. Amarger1383 γ P. GrahamBC15 α N. Amarger1385 γ P. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,Ec4A δ G. BernalWater and Environmental Science, University of Arizona, Tucson; P. Graham,Ec101 γ G. BernalRhizobium Research Laboratory, University of Minnesota, St. Paul; E. Mar-Ec20A δ G. Bernalinez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex-ico; G. Bernalico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-ico; G. Bernalico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-ico; G. Bernalico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-ico; G. Bernaliso, Estación Experimental Santa Catalina, Quito, Ecuador; M. Hungría,1468 α P. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri-1478 α P. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri-	1002	γ	P. Graham	ISP-69	γ	D. Rodriguez-Navarro (23)	
EcuadorGD131 γ N. Amarger1373 δ P. GrahamGD164 γ N. Amarger1374 α P. GrahamGD261 γ N. Amarger1376 δ P. Graham (8)CP91 γ N. Amarger1377 δ P. Graham (8)Burundi1378 γ P. GrahamBC15 α N. Amarger1383 γ P. GrahamBC15 α N. Amarger1385 γ P. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,Ec4A δ G. BernalRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,Ec101 γ G. BernalKater and Environmental Science, University of Arizona, Tucson; P. Graham,Ec20A δ G. Bernaltínez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex-Ec21 γ G. Bernalco; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-Ec24A δ G. Bernalco; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-ias, Estación Experimental Santa Catalina, Quito, Ecuador; M. Hungría,1468 α 1478 α P. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri-	1640	γ	P. Graham	Indonesia			
1373δP. GrahamGD164γN. Amarger1374αP. GrahamGD261γN. Amarger1376δP. Graham (8)CP91γN. Amarger1377δP. Graham (8)BurundiBT1378γP. Graham (8)BC15αN. Amarger1383γP. GrahamBC15αN. Amarger1385γP. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,Ec4AδG. BernalWater and Environmental Science, University of Arizona, Tucson; P. Graham,Ec101γG. BernalRhizobium Research Laboratory, University of Minnesota, St. Paul; E. Mar-Ec20AδG. Bernalinez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex-Ec21γG. Bernalico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-ias, Etación Experimental Santa Catalina, Quito, Ecuador; M. Hungría,ias, Estación Experimental Soia, Embrapa, Londrina, Brazil; P. Lezama, Universidad1478αP. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri-	Ecuador			GD131	γ	N. Amarger	
1374αP. GrahamGD261γN. Amarger1376δP. Graham (8)CP91γN. Amarger1377δP. Graham (8)Burundi1378γP. Graham (8)BC15αN. Amarger1383γP. GrahamBC15αN. Amarger1385γP. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,Ec4AδG. BernalWater and Environmental Science, University of Arizona, Tucson; P. Graham,Ec101γG. BernalKhizobium Research Laboratory, University of Minnesota, St. Paul; E. Mar-Ec20AδG. Bernaltínez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex-Ec21LγG. Bernalis, Estación Experimental Santa Catalina, Quito, Ecuador; M. Hungría,Ec24AδG. Bernalias, Estación Experimental Soi, Embrapa, Londrina, Brazil; P. Lezama, Universidad1468αP. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri-1478αP. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri-	1373	δ	P. Graham	GD164	γ	N. Amarger	
1376δP. Graham (8)CP91γN. Amarger1377δP. Graham (8)Burundi1378γP. Graham (8)BC15αN. Amarger1383γP. GrahamBC15αN. Amarger1383γP. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil, Recherche Agronomique, Dijon, France; K. Josephson, Department of Soil, Vater and Environmental Science, University of Arizona, Tucson; P. Graham, Recherche Agronomique, Dijon, France; K. Josephson, Department of Soil, Vater and Environmental Science, University of Arizona, Tucson; P. Graham, Rhizobium Research Laboratory, University of Minnesota, St. Paul; E. Mar- tínez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex- ico; G. BernalEc2LγG. BernalEc24AδG. BernalL68αP. Graham (8)4178αP. Graham (8)	1374	α	P. Graham	GD261	γ	N. Amarger	
1377δP. Graham (8)Burundi1378γP. Graham (8)BC15αN. Amarger1383γP. GrahamBC15αN. Amarger1385γP. Graham*N. Amarger, Laboratoire de Microbiologie des Sols, Institut Nacional de la1385γP. GrahamRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,Ec4AδG. BernalWater and Environmental Science, University of Arizona, Tucson; P. Graham,Ec10γG. BernalRhizobium Research Laboratory, University of Minnesota, St. Paul; E. Mar-Ec20AδG. Bernaltínez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex-Ec21γG. Bernalico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-Ec24AδG. Bernalias, Estación Experimental Santa Catalina, Quito, Ecuador; M. Hungría,1468αP. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri-1478αP. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri-	1376	δ	P. Graham (8)	CP91	γ	N. Amarger	
1378γP. Graham (8)BC15αN. Amarger1383γP. Graham*N. Amarger, Laboratoire de Microbiologie des Sols, Institut Nacional de la1385γP. Graham*N. Amarger, Laboratoire de Microbiologie des Sols, Institut Nacional de la1385γP. Graham*N. Amarger, Laboratoire de Microbiologie des Sols, Institut Nacional de laEc4AδG. BernalRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil, Water and Environmental Science, University of Arizona, Tucson; P. Graham, Ec101Ec101γG. BernalKhizobium Research Laboratory, University of Minnesota, St. Paul; E. Mar- tínez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex- ico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar- ias, Estación Experimental Santa Catalina, Quito, Ecuador; M. Hungría, 14681478αP. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri- cultura Tropical	1377	δ	P. Graham (8)	Burundi			
1383γP. Graham1385γP. Graham1385γP. Graham1385γP. GrahamEc4AδG. BernalEc6AδG. BernalEc101γG. BernalEc20AδG. BernalEc2LγG. BernalEc24AδG. BernalEc24AδG. BernalEc24AδG. BernalIc24AδG. Bernal	1378	γ	P. Graham (8)	BC15	α	N. Amarger	
1385γP. Graham*N. Amarger, Laboratoire de Microbiologie des Sols, Institut Nacional de la Recherche Agronomique, Dijon, France; K. Josephson, Department of Soil, Water and Environmental Science, University of Arizona, Tucson; P. Graham, Ec101Ec101γG. BernalWater and Environmental Science, University of Arizona, Tucson; P. Graham, Rhizobium Research Laboratory, University of Minnesota, St. Paul; E. Mar- tínez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex- ico; G. BernalEc20AδG. Bernalico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar- ico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar- ias, Estación Experimental Santa Catalina, Quito, Ecuador; M. Hungría, 14681478αP. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri- cultura Tropical	1383	γ	P. Graham				
Ec4AδG. BernalRecherche Agronomique, Dijon, France; K. Josephson, Department of Soil,Ec6AδG. BernalWater and Environmental Science, University of Arizona, Tucson; P. Graham,Ec10IγG. BernalRhizobium Research Laboratory, University of Minnesota, St. Paul; E. Mar-Ec20AδG. Bernaltínez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex-Ec2LγG. Bernalico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-Ec24AδG. Bernalias, Estación Experimental Santa Catalina, Quito, Ecuador; M. Hungría,1468αP. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri-1478αP. Graham (8)Cultura Tropical	1385	γ	P. Graham	*N. Amarger, Laboratoir	e de Microbiologie	e des Sols, Institut Nacional de la	
EchδG. BernalWater and Environmental Science, University of Arizona, Tucson; P. Graham,Ec6AδG. BernalRhizobium Research Laboratory, University of Minnesota, St. Paul; E. Mar-Ec101γG. Bernaltinez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex-Ec20AδG. Bernalico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-Ec21γG. Bernalias, Estación Experimental Santa Catalina, Quito, Ecuador; M. Hungría,Ec24AδG. BernalCentro Pesquisa Soja, Embrapa, Londrina, Brazil; P. Lezama, Universidad1468αP. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri-1478αP. Graham (8)Centro Pesquisa Londrina, Pracial	Fc4A	δ	G Bernal	Recherche Agronomiqu	ie, Dijon, France; K	. Josephson, Department of Soil,	
LtoroG. BernalRhizobium Research Laboratory, University of Minnesota, St. Paul; E. Mar- tínez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex- ico; G. BernalEc20AδG. Bernaltínez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex- ico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar- ias, Estación Experimental Santa Catalina, Quito, Ecuador; M. Hungría, 14681478αP. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri- cultura Tropical	EchA	8	G. Bernal	Water and Environment	al Science, Universi	ty of Arizona, Tucson; P. Graham,	
EctorγG. Bernaltínez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex-Ec20AδG. Bernalico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar-Ec2LγG. Bernalias, Estación Experimental Santa Catalina, Quito, Ecuador; M. Hungría,Ec24AδG. BernalCentro Pesquisa Soja, Embrapa, Londrina, Brazil; P. Lezama, Universidad1468αP. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri-	ECOA	0	G. Bernal	Rhizobium Research La	boratory, Universit	y of Minnesota, St. Paul; E. Mar-	
EC20AφG. Bernalico; G. Bernal, Instituto Nacional Autónomo de Investigaciones Agropecuar- ias, Estación Experimental Santa Catalina, Quito, Ecuador; M. Hungría, Centro Pesquisa Soja, Embrapa, Londrina, Brazil; P. Lezama, Universidad1468αP. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri- cultura Tropical		γ	G. Dernal	tínez-Romero, Centro sobre Fijación de Nitrógeno, UNAM, Cuernavaca, Mex-			
EC2LγG. Bernalias, EstaciónExperimental Santa Catalina, Quito, Ecuador; M. Hungría,Ec24AδG. Bernalias, EstaciónExperimental Santa Catalina, Quito, Ecuador; M. Hungría,1468αP. Graham (8)Centro Pesquisa Soja, Embrapa, Londrina, Brazil; P. Lezama, Universidad1478αP. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri-		δ	G. Bernal	ico; G. Bernal, Instituto I	Nacional Autónom	o de Investigaciones Agropecuar-	
EC24AδG. BernalCentro Pesquisa Soja, Embrapa, Londrina, Brazil; P. Lezama, Universidad1468αP. Graham (8)Privada Antenor Orrego, Trujillo, Perú; CIAT, Centro Internacional de Agri-1478αP. Graham (8)Cultura Tropical	EC2L	γ	G. Bernal	ias, Estación Experimental Santa Catalina. Ouito. Ecuador: M. Hungría.			
1468 α P. Graham (8) Centro i respina Soja, Entropo, E	Ec24A	δ	G. Bernal	Centro Pesquisa Sola	mbrana. Londrina	Brazil: P. Lezama Universidad	
1478 α P. Graham (8) Culture Tropical	1468	α	P. Graham (8)	Privada Antenor Orrego, Trujillo, Perú: CIAT. Centro Internacional de Agri-			
	1478	α	P. Graham (8)	cultura Tropical		, centro internacional de Agn-	

demonstrated that the RFLP analysis of the *nod*C gene proved to be useful as a molecular marker for the identification of *R. etli* lineages associated with each center of bean domestication. In this study we analyzed the *R. etli* populations from various geographical origins, mainly from American BD centers, and found that each *nod*C type has a markedly distinct geographical distribution. The discovery that one lineage characterized by *nod*C type δ that was prevalent among the populations isolated from NWA was also present among isolates from Ecuador and Peru prompted us to examine the soils, as well as wild and cultivated beans, from the regions that had been proposed as centers of bean diversity and domestication. The finding that *R. etli* populations retrieved from Mexican soils were clearly different from those retrieved from soils from Ecuador, Bolivia, and Argentina was one of the most significant features of this research, indicating that the distribution of *R. etli* lineages follows that of centers of bean diversity and domestication. *R etli* lineages characterized by the presence of *nod*C alleles types α and γ were

UNAS PNAS PNAS P

Table 2. Results from nodC typing of R. etli isolates from soils of
Mexico Ecuador, Bolivia, and Argentina retrieved with the
indicated bean accession

	Bean accession	Nodule occupancy by <i>nod</i> C type				
Origin of soil		n	α	β	γ	δ
Mexico	Mesoamerican					
	Negro Xamapa	48	30	0	18	0
	G2771	20	20	0	0	0
	G12866	-	-	-	-	-
	Andean					
	G19890	56	39	0	17	0
	G4474	48	36	0	12	0
	G21245*	38	35	0	3	0
	G21197	51	23	0	28	0
	Wild beans NWA	38	13	0	25	0
Ecuador	Mesoamerican					
	Negro Xamapa	73	35	0	38	0
	G2771	-	-	-	-	-
	G12866	14	11	0	0	3
	Andean					
	G19890	27	7	0	12	8
	G4474	65	21	6	36	2
	G21245*	-	-	-	-	_
	G21197	29	10	0	19	0
	Wild beans NWA	-	-	-	-	_
Bolivia	Mesoamerican					
	Negro Xamapa	26	1	0	0	25
	G2771	-	-	-	-	-
	G12866	-	-	-	-	_
	Andean					
	G19890	-	-	-	-	_
	G4474	-	-	-	-	_
	G21245*	-	-	-	-	_
	G21197	17	0	0	0	17
	Wild beans NWA	35	0	0	0	35
NWA	Mesoamerican					
	Negro Xamapa	45	0	11	0	34
	G2771	32	0	4	0	28
	G12866	_	_	_	_	_
	Andean					
	G19890	49	0	13	0	36
	G4474	_	_	_	_	_
	G21245*	_	_	-	_	_
	G21197	56	0	11	0	45
	Wild beans NWA	22	0	0	0	22

NWA, northwest Argentina; –, not determined. *Representative of the intermediate gene pool (5).

found in the Mesoamerican soil sample from Mexico, whereas lineage *nod*C type δ was found in soils from Ecuador and from the southern Andes in Bolivia and Argentina. It is therefore interesting to observe that *nod*C allele δ is found geographically restricted to the southern Andes regions and Ecuador. The area of Ecuador is considered intermediate between that of the Mesoamerican and Andean bean gene pools (5). It is noteworthy that *nod*C alleles β and δ were also identified as the only *nod*C alleles found among the very limited number of bean-retrieved isolates carrying the R. leguminosarum bv. phaseoli 16S rRNA allele and that *nod*C type β was found tightly associated with the 16S rRNA allele of R. leguminosarum. Whether in R. leguminosarum the allele nodC type β evolved from the allele nodC type δ after lateral transfer from *R. etli* or, conversely, the *nod*C type δ arose in *R. leguminosarum* from the type β and afterward transferred to R. etli remains to be investigated.

To test whether lineages that were recovered at high frequency from each soil sample are more competitive for nodulation or are just better adapted to soil and numerically dominant, assays for nodulation competitiveness were performed with representative

Table 3. Results of coinoculation of beans from Mesoamerica and southern Andes using a mixture of *nod*C type α and type δ strains

Region	N*	Nodule occupancy by <i>nod</i> C type δ,† %
Mesoamerican Mexico, El Salvador, Guatemala, and Colombia	460	3 ± 2.4
Andean Ecuador, Peru, Bolivia, Chile, and Argentina	1,021	37 ± 10

*Number of nodules examined.

[†]Range of means at P = 0.05 by Student's t test.

bean plants from the different genetic pools. Although several studies have used agriculturally cultivated beans (8, 26), our study was performed mostly with wild common beans, and its resulting data were quantitative and suitable for statistical analysis. We found accessions of wild as well as cultivated common beans that are preferentially nodulated by the lineage *nod*C type α , whereas other accessions nodulate preferentially with the lineage *nod*C type δ . Overall, the occupancy of the *nod*C type δ strain in nodules of Andean beans was significantly higher than in those of Mesoamerican beans; the reverse was true for *nod*C type α strains. Moreover, this result and the distribution and abundance of the two rhizobial types in soils of both regions were in agreement. Therefore, these results seem to reveal that a particular R. etli lineage is not only more abundant in soils from a given region but also has a certain affinity for plants collected in the same region. Together, these observations may result in a synergism in which, during the growth cycle of beans, the more competitive rhizobia will turn into the soil after nodule senescence and therefore contribute to an increase in their representation in soil. Furthermore, from the point of view of the plant and the microsymbiont as well, this result suggested the presence of genes in beans and in rhizobia that determine affinity between plant and rhizobia and also suggested that geographical isolation led to some specificity. It is remarkable that in these coinoculation experiments an almost exclusive nodule occupation by *nod*C type α occurs in the case of Mesoamerican plants. This observation suggests that the selectivity for *nod*C type α is a common trait among Mesoamerican bean populations. Because most of the accessions we have examined had been characterized previously, we were able to extend comparisons to other traits, such as the phaseolin type (4, 5, 22, 24). Accessions showing affinity for lineage *nod*C type α have mostly S and M variants of phaseolin, whereas those accessions having significant nodulation with lineage nodC type δ were characterized by having the T-, C-, Pa-, and I-type phaseolin. This result indicated that our observations still are valid for the variability present within each genetic pool. Analysis of R. etli populations from regions that were the target of bean dissemination, such as Spain, has shown *nod*C allele types α and γ . Gepts and Bliss (23) and Ocampo et al. (22) have examined Spanish bean cultivars and found that phaseolin type C was detected at the highest frequency, followed by type T, type S, and type H, indicating that most Spanish beans share the south Andean gene pool, therefore suggesting that Spanish bean domestication may have developed from materials introduced from the Andean zone. Assuming that rhizobia are indeed carried by seeds, it appears that only two types among the three lineages of R. etli we found in the Americas are currently detected in Spain: α and γ . It is possible that only those two lineages survived desiccation stress on seeds (27) or, alternatively, were successful in competition and adaptation to new environments.

Our results invite speculation that each of the major genetic pools of *P. vulgaris* and rhizobia coevolved independently of the others after geographic separation. Arguments in favor of this hypothesis

are our data on the characterization of R. etli strains isolated from different regions of the Americas that show that the *nod*C type of rhizobia in populations from the Andean region is different from those isolated from the Mesoamerican region; they may represent evolutionarily distinct lineages. The high prevalence of nodC type δ among the *R. etli* isolates from wild common beans in NWA may reflect the environmental isolation in which the Argentinean wild bean populations are found (2).

By using analysis of DNA fingerprinting, Bernal and Graham (8) examined a collection of bean rhizobial isolates from Mexico and Ecuador, some of which are also investigated in this work, and found three clusters that corresponded to isolates from Mexico and the northern and southern Andean regions, respectively, providing additional evidence of the diverse genetic structures of R. etli populations in Latin America.

Our previous results, as well as data from other investigators, indicate that R. etli is the species most commonly associated with beans in the Americas; therefore, the presumed coevolution we propose here represents an event that might have taken place after P. vulgaris and Rhizobium spp. coevolved symbiotically toward species R. etli. The distinct organization of R. etli common nodulation genes, in which, unlike in most rhizobial species, nodA is separated from the nodBC genes (28), could be considered a molecular marker ("remains") of the early evolutionary process of "gathering" specific symbiotic genes. Afterward, diversification of species R. etli in geographically separated and isolated areas might have occurred.

Coevolution between host plant and microorganisms has been shown in pathogenic interactions in beans and in rhizobia-legume interactions. Geffroy et al. (6) performed cross-inoculations between common bean plants and the fungal pathogen Colletotrichum lindemuthianum, which was collected in the three BD centers, and found that coevolution led to differentiation of host resistance in the three centers of domestication, with each plant population showing resistance against allopatric strains. A typical example of rhizobia-legume coevolution is that described by Lie et al. (29), in which the pea cultivar from Afghanistan is nodulated by rhizobia isolated from soils of the Middle East and Central Asia but not by the European R. leguminosarum strains. These two cases are examples of coevolution due to geographical isolation, which might also have been the situation in the common bean-rhizobia interaction. But what could be the basis for this host plant-rhizobia interaction? In the present work, the diverse lineages of R. etli have been defined according to their distinct RFLP profile of the *nod*C gene, actually due to the presence of the HinfI polymorphism. The nodC gene product is an N-acetyl-glucosaminyltransferase, which is involved in the first step in Nod-factor assembly (30, 31). We have

Becerra Velásquez, V. L. & Gepts, P. (1994) Genome 37, 256-263.

- Beebe, S., Rengifo, J., Gaitan, E., Duque, M. C. & Tohme, J. (2001) Crop Sci. 41, 854-862. 3. Singh, S. P., Gepts, P. & Debouck, D. G. (1991) Econ. Bot. 45, 379-396.
- Tohme, J., Gonzalez, D. O., Beebe, S. & Duque, M. C. (1996) *Crop Sci.* 36, 1375–1384. Kami, J., Becerra Velásquez, V., Debouck, D. G. & Gepts, P. (1995) *Proc. Natl. Acad. Sci.* 5. USA 92, 1101-1104.
- Geffroy, V. D., Sicard, D., de Oliveira, J. C. F., Sevignac, M. S., Cohen, S., Gepts, P., Neema, C., Langin, T. & Dron, M. (1999) *Mol. Plant–Microbe Interact.* 12, 774–784.
 Amarger, N. (2001) *Adv. Agron.* 73, 109–168.
- Bernal, G. & Graham, P. H. (2001) Can. J. Microbiol. 47, 526-534
- Montealegre, C. & Kipe-Nolt, J. (1994) Arch. Microbiol. 162, 352–356.
 Rodríguez-Navarro, D. M., Buendía, A. M., Camacho, M., Lucas, M. M. & Santamaría, C.
- (2000) Soil Biol. Biochem. 32, 1601-1613. 11. Sessitsch, A., Hardarson, G., Akkermans, A. D. & de Vos, W. M. (1997) Mol. Ecol. 6,
- 601-608. 12. Silva, C., Vinuesa, P., Eguiarte, L. E., Martínez-Romero, E. & Souza, V. (2003) Appl.
- Environ. Microbiol. 69, 884-893. 13. Aguilar, O. M., López, M. V., Riccillo, P. M., González, R. A., Pagano, M., Grasso, D. H.,
- Pühler, A. & Favelukes, G. (1998) Appl. Environ. Microbiol. 64, 3520-3524.
- Aguilar, O. M., López, M. V. & Riccillo, P. M. (2001) J. Biotechnol. 91, 181–188.
 Vincent, J. M. (1970) A Manual for the Practical Study of the Root-Nodule Bacteria (IBP)
- Handbook No. 15) (Blackwell Scientific, Oxford).
- Alippi, A. M. & Aguilar, O. M. (1998) *J. Inverterbr. Pathol.* **72**, 21–27.
 Laguerre, G., Nour, S. M., Macheret, V., Sanjuan, J., Drouin, P. & Amarger, A. (2001) Microbiology 147, 981-993.

examined the nucleotide sequence of nodC from strains that represent each lineage of R. etli and found that the polymorphism arises from single nucleotide transversions at positions within the HinfI recognition site that do not affect the translational reading frame and represent neutral amino acid changes (data not shown). Therefore, it is unlikely that this nucleotide polymorphism in nodC causes functional changes in the NodC protein or changes in the plant-rhizobia interaction. Rather, we believe that the nodC profile is a molecular marker associated with a certain affinity for beans from each BD center, the basis of which remains to be discovered. Interestingly, R. etli strains belonging to each of the lineages are able to nodulate beans from the different centers of diversity and domestication, arguing against a situation of restricted nodulation such as is found with certain soybeans, peas, and beans. For instance, Sinorhizobium fredii strain USDA191 forms nodules on the soybean cultivars McCall and Peking, but S. fredii strain USDA257 nodulates only cultivar Peking (32). The Afghanistan pea cultivar is resistant to nodulation by European R. leguminosarum strains but is efficiently nodulated by strains isolated from Israel (29). Further investigations demonstrated that the ability to acetylate the nodulation factor introduced into the European strains lifts the restriction and makes them able to nodulate cv. Afghanistan (33). In contrast to this, in our case the absence of absolute restriction limits to cross-nodulation is clearly marked by the effective nodulation of bean accessions by allopatric R. etli strains (*nod*C type δ strains nodulating Mesoamerican accessions, and its reverse, type α strains forming nodules with Andean accessions; Table 2), and the existence of strong sympatric competition effects (Table 3) demonstrates nodulation preference between lineages of R. etli and beans from the same region of diversity.

Although our results provide no evidence for the basis and mechanisms underlying this nodulation preference, we find them provocative. It would be of interest to determine whether features we found in this work are encoded by the symbiotic plasmid in each lineage, because it was shown that symbiotic genes in R. etli are borne in plasmids (20). It also will be of interest to pursue investigations of the bean genetic background involved in this trait in the plant. Overall, these findings might offer clues for applied aspects of the bean-rhizobia symbiosis, such as a search for strains with better chances to compete against indigenous strains in a program of bean inoculation.

We thank all the people who provided samples of soil, seeds, and strains and Gabriel Favelukes for his critical revision of the manuscript. O.M.A. is a member of and O.R. is the recipient of a training studentship from Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina.

- 18. Steel, R. G. D. & Torrie, J. H. (1990) Principles and Procedures of Statistics: A Biometrical Approach (McGraw-Hill, New York).
- 19. Amarger, N., Macheret, V. & Laguerre, G. (1997) Int. J. Syst. Bacteriol. 47, 996-1006.
- Brom, S., Girard, L., García-de los Santos, A., Sanjuán-Pinilla, J. M., Olivares, J. & Sanjuán, J. (2002) Appl. Environ. Microbiol. 69, 2555–2561.
 Michiels, J., Van Soom, T., D'hooghe, I., Dombrecht, B., Benhassine, T., de Wilde, P. &
- Vanderleyden, J. (1998) J. Bacteriol. 180, 1729–1740.
 Ocampo, C. H., Martin, J. P., Ortiz, J. M., Sánchez-Yélamo, M. D., Toro, O. & Debouck, D. G. (2002) Annu. Rep. Bean Improv. Coop. 45, 236–238.
- Gepts, P. & Bliss, F. A. (1988) Econ. Bot. 42, 86-104.
- Gepts, P. (1990) Econ. Bot. 44, 28–38.
 Chaverra, M. H. & Graham, P. H. (1992) Crop Sci. 32, 1432–1436.
- 26. Graham, P. H., Rosas, J. C., Estevez de Jensen, C., Peralta, E., Tlusty, B., Acosta-Gallegos, J. & Arraes Pereira, P. A. (2003) Field Crops Res. 82, 179–192.
 Pérez-Ramírez, N. O., Rogel, M. A., Wang, E., Castellanos, J. Z. & Martínez-Romero, E.
- (1998) FEMS Microbiol. Ecol. 26, 289-296
- 28. Vázquez, M., Dávalos, A., de las Peñas, A., Sánchez, F. & Quinto, C. (1991) J. Bacteriol. 173, 1250-1258
- Lie, T. A., Goktan, D., Egin, M., Pijnenborg, J. & Anlarsal, E. (1987) *Plant Soil* 100, 171–181.
 Long, S. R. (2001) *Plant Physiol.* 125, 69–72.
 Perret, X., Staehelin, C. & Broughton, W. J. (2000) *Microbiol. Mol. Biol. Rev.* 64, 180–201.
- Pueppke, S. G., Bolaños-Vasques, M. C., Werner, D., Bec-Forté, M.-P., Promé, J.-C. & Krishnan, H. B. (1998) *Plant Physiol.* 117, 599–606.
- 33. Firmin, J. L., Wilson, K. E., Carlson, R. W., Davies, A. E. & Downie, J. A. (1993) Mol. Microbiol. 10, 351-360