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ETHNOMICROBIOLOGY: DO AGRICULTURAL PRACTICES MODIFY THE POPULATION STRUCTURE OF THE NITROGEN FIXING BACTERIA *RHIZOBIUM ETLI* BIOVAR *PHASEOLI*?

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ABSTRACT.—We analyze how agricultural practices affect the levels of genetic variation and the genetic structure of beans (*Phaseolus vulgaris* and *P. coccineus*) and of their associated bacteria *Rhizobium etli*. Two contrasting communities in the state of Puebla, central Mexico, were selected for this study: San Miguel, a Nahuatl community where traditional agricultural work is done almost exclusively by women, and Calpan, where men cultivate crops using modern techniques. The results are compared with previous research from Morelos, also in central Mexico. We found that San Miguel has maintained its agricultural tradition for generations. In recent years, women have played an important role not only in preserving this tradition but also in conserving the biological diversity in their plots. In Calpan, by contrast, the local varieties of beans have been replaced by commercial varieties and the women participate minimally in agriculture. In general terms, the genetic diversity of *R. etli* associated with cultivated beans (both *P. vulgaris* and *P. coccineus*) is high in all the communities we studied, while it is lower for the rhizobia associated with wild beans. The population structure of *Rhizobium etli* is different in the two communities: the most fertile and intensively managed plots are similar in this respect, while the least managed plots resemble the site of wild *P. vulgaris*. This research indicates that agricultural practices and local environmental conditions affect the genetic structure of both cultivated beans and their associated bacteria.

RESUMEN.—Analizamos cómo las prácticas agrícolas afectan los niveles de variación genética y la estructura genética de los frijoles (*Phaseolus vulgaris* y *P. coccineus*) y de sus bacterias asociadas, *Rhizobium etli*, y el papel de las mujeres en la conservación y manejo de esta diversidad genética. Se seleccionaron dos comunidades contrastantes en el estado de Puebla, en el centro de México: San Miguel, una comunidad náhuatl donde el trabajo agrícola tradicional lo llevan a cabo casi exclusivamente mujeres, y Calpan, una comunidad mestiza donde los hombres cultivan el campo usando técnicas modernas. Los resultados se comparan con investigaciones previas realizadas en Morelos, también en el centro de México. Encontramos que San Miguel ha mantenido su tradición agrícola por generaciones. En años recientes, las mujeres han jugado un papel importante no sólo preservando esta tradición sino conservando la diversidad biológica en sus parcelas. Sin embargo, en Calpan las variedades locales de frijol han sido substituídas por variedades comerciales y las mujeres están perdiendo sus tradiciones y su contacto con la tierra. En términos generales, la diversidad genética de *R. etli* asociado a frijoles cultivados (tanto *P. vulgaris* como *P. coccineus*) es alta en todas las comunidades estudiadas, mientras que para los rhizobia asociados a frijoles silvestres es menor. La estructura poblacional de *Rhizobium etli* es diferente en las distintas comunidades estudiadas, siendo las parcelas más fértiles y más intensivamente cultivadas similares entre sí, mientras que las parcelas manejadas menos intensivamente se parecen al sitio de *P. vulgaris* silvestre. Este trabajo indica que las prácticas agrícolas, la influencia de las mujeres y las condiciones ambientales locales afectan la estructura genética tanto de los cultivos como de sus bacterias asociadas.

RÉSUMÉ.—Dans cet article, nous analysons l'effet des pratiques agricoles sur les niveaux de variation génétique et la structure génétique des haricots (*Phaseolus vulgaris* et *P. coccineus*) et des bactéries (*Rhizobium etli*) qui leur sont associées et le rôle des femmes dans la conservation et la gestion de cette diversité génétique. Deux communautés contrastantes de l'état de Puebla dans le Mexique central ont été sélectionnées: San Miguel, une communauté nahuatl où le travail agricole traditionnel est du ressort presqu'exclusif des femmes, et Calpan, une communauté métisse où ce sont les hommes qui pratiquent l'agriculture avec des techniques modernes. Nous avons comparé nos résultats avec une autre étude que nous avions menée à Morelos qui est aussi situé dans le centre du Mexique. Nous avons découvert que San Miguel a maintenu ses traditions agricoles à travers les générations. Dans la période récente, les femmes ont joué un rôle important non seulement en préservant ces traditions mais également dans la conservation de la diversité biologique sur leurs lopins de terre. Toutefois, à Calpan, les variétés locales de haricots ont été remplacées par les variétés commerciales et les femmes sont en train de perdre leurs traditions et leur rapport à la terre. Dans l'ensemble, la diversité génétique de la bactérie *R. etli* qui est associée aux haricots (autant à *P. vulgaris* qu'à *P. cocineus*) est élevée dans toutes les communautés étudiées, bien qu'elle le soit moins dans le cas des rhizobiums associés aux haricots sauvages. La structure de la population de *Rhizobium etli* est différente selon les communautés étudiées, les lots les plus fertiles et les mieux gérés étant similaires tandis que les lots les moins bien gérés se rapprochant davantage des sites sauvages de *P. vulgaris*. Cette recherche montre que les pratiques agricoles, l'influence des femmes et les conditions environnementales locales affectent la structure génétique à la fois des récoltes et des bactéries associées.

INTRODUCTION

Mexico is a country with an enormous cultural and biological richness (Ramamoorthy *et al.* 1993; Flores-Villela and Gerez 1994). This legacy is being lost at an unprecedented rate because of increasing demographic, economic, and technological pressures. In order to preserve crop diversity, it is necessary to understand the relationship between the human management of these species and their genetic diversity. The process of domestication is an ideal system to understand the influence of humans over biological diversity (Doebley 1989). Usually, the domestication process erodes the natural genetic diversity of the organisms under domestication (Doebley 1989; Escalante *et al.* 1994). However, relatively high levels of genetic variation can be maintained as landraces by indigenous cultures (Kaplan 1981; Brush 1986; Altieri and Merrick 1987; Doebley 1989). It has been suggested that rural women play an important role in the preservation of genetic diversity by their use of traditional knowledge of agricultural practices as well as by the use of seeds with "old" genotypes (Brush 1986; Bain 1993).

The common bean, *Phaseolus vulgaris*, is an ancient crop species. Domesticates appear in the archeological record 7,000 B.P. in both Mesoamerica and South America (Gentry 1969; Kaplan 1981; Delgado *et al.* 1988). In Mexico, the common bean and the related cultivated perennial species *P. coccineus*, are normally nodulated by strains of the nitrogen-fixing bacteria, *Rhizobium etli* (Segovia *et al.* 1993; Souza *et al.* 1994). Beans, as most legumes, allow certain strains of *Rhizobium* to penetrate into their roots and subsequently to develop nodules where nitrogen fixation occurs. The nodule structure and the transformation of the atmospheric nitrogen to ammonia is an active process mediated by the plant as well as the bacteria in the nodules, representing one of the clearest examples of symbiosis (Long 1989). Nitrogen fixation efficiency, number of nodules, and their size depend on the correct molecular signals between both symbiotic partners (Long 1989; Souza 1990). Traditionally, the evolutionary biology of the plant host and the bacteria has been studied as two separate entities without considering human influence on their genetic diversity, even though there is evidence that humans have contributed to the spread of rhizobia around the world by transporting seeds and soil from one continent to the other (Martínez-Romero and Caballero Mellado 1996).

Population genetics of wild and cultivated *P. vulgaris* and *P. coccineus* from central Mexico has been studied by Piñero and Eguiarte (1988) and Escalante *et al.* (1994). *P. coccineus* from central Mexico has a high genetic diversity (measured as *H*, the mean expected heterozygosity in Hardy Weinberg equilibrium, range 0.18-0.27) and intermediate outcrossing rates (*t* range 0.59 to 0.69, Escalante *et al.* 1994). In this species, the domestication process has neither eroded the levels of genetic variation nor changed the mating system (Escalante *et al.* 1994). *P. vulgaris*, in contrast, is highly inbred (almost entirely self-pollinated), and has very low levels of genetic variation ($H = 0.041$; Escalante *et al.* 1994).

The population genetics of *Rhizobium* sp. have been studied by several authors (Piñero *et al.* 1988; Demezaz *et al.* 1991; Segovia *et al.* 1991; Souza *et al.* 1992, 1994; Eardly *et al.* 1990 1995; Strain *et al.* 1995), who have found high levels of genetic diversity in *Rhizobium* associated with cultivated legumes (*H* ranges from 0.46 to 0.69). Souza *et al.* (1994) described similar results in the rhizobia associated with cultivated beans of Morelos in central Mexico ($H = 0.41$). However, the rhizobia associated with wild *P. vulgaris* in Morelos presented a much lower genetic diversity ($H = 0.11$).

The main objectives of this research were to analyze how agricultural practices affect the genetic diversity of crops and of the bacteria associated with them, and to explore the role of women in the conservation and management of the genetic diversity of beans and their rhizobia. To achieve these objectives, two contrasting communities in the central Mexican state of Puebla were selected: San Miguel Acuexcomac, a Nahuatl community where traditional agricultural work is done almost exclusively by women, and San Andrés Calpan, a Mestizo community where men cultivate the fields using modern techniques. The results were compared with those of previous research from Tepoztlan and Santiago Tepetlapa, Morelos (Souza 1990; Souza *et al.* 1994). In both communities we studied the role of women in agriculture and the population genetics of cultivated beans (*Phaseolus vulgaris* and *P. coccineus*) and nitrogen fixing bacteria *Rhizobium etli*. This is the first study where the interaction between the beans and the nitrogen fixing bacteria is analyzed in different agrosystems with the purpose of understanding the effect of plant and soil management on the bacteria.

METHODS

Study Sites.—The sites where this research was carried out are located near Mexico City, in the highlands of central Mexico, their general characteristics are described in Table 1. We chose these communities because one (San Miguel) presented the lowest bean production registered in the state of Puebla, while Calpan has one of the highest yields in the state (Table 2) (INEGI 1994; M. Colunga and D. Piñero personal communication).

TABLE 1—General characteristics of the study communities in Puebla and Morelos, Mexico.

Characteristics/Sites	**Calpan, Puebla**	**Santiago, Morelos**[b]	**San Miguel, Puebla**[c]
Climate[a]	temperate	subtropical	semi-arid
Elevation[a]	2,500 m	1,500 m	2,100 m
Coordinates[a]	99°30′W, 19°05′N	99°05′W, 18°59′N	98° 05′W, 18°50′N
Vegetation[a]	oak and pine forest	tropical deciduous forest	scrubland
Rainfall[a]	800-1,000 mm	1,000 mm	500-600 mm
Soil management	tractor, hand and chemical weeding	hand weeding	minimal tilling and hand weeding
Agrochemical use	fertilizer, pesticide	fertilizer, pesticide	low levels of fertilizer
Soil structure and fertility	sandy clay loam, sandy loam; fertile	sandy clay loam; fertile	clay, sandy clay loam; eroded
Soil pH	6.2	5.5	8.3
Culture	acculturated, Nahuatl no longer spoken	rural, Nahualt	rural, Nahuatl
Family	male dominant	male dominant	men are absent 75% of the time
Cultivars in same plot	bean plots separated by a row of fruit trees	beans	many bean varieties, corn, squash, and associated greens
Average number of plant spp./plot	5-7 species/plot	not studied	16-21 species/plot

[a] Zamora Rodríguez 1989; Inegi 1994
[b] Souza 1990; Souza *et al.* 1994
[c] Niehe 1988; INEGI 1994

San Miguel Acuexcomac, Puebla (hereafter San Miguel), is an indigenous (Nahuatl) community in the municipality of Cuautinchán near the Valsequillo Dam. The land in San Miguel has been marginal for agriculture since pre-Columbian times. Agriculture is mainly a polyculture system, cultivating corn, beans, squash, chili, and different greens. At present, the land in San Miguel is cultivated mainly by women, since most of the men are working in Los Angeles, California (Niehe 1988; V. Souza personal observation). The soil has low levels of soluble nitrogen (nitrates), and a moderate alkalinity (Figure 1); rainfall is lower and more irregular than in Calpan (Niehe 1988).

San Andrés Calpan, Puebla (hereafter Calpan) is the seat of a municipality close to Cholula and Huejotzingo. Calpan is an acculturated community where the indigenous language is no longer spokey. Here, the capitalization process and the input of mechanized technology have allowed an increase of agricultural productivity (Table 1) (Zamora Rodriguez 1989), but at a high cultural and biological price: agricultural traditions as well as germ plasm are being lost. In Calpan, the land is cultivated mostly by men with the help of their families (V. Souza personal observation). The soil has more soluble nitrogen and a more balanced pH than San Miguel, making it more fertile (Figure 1); Calpan has a more predictable rain pattern and a more temperate climate than San Miguel because of the shadow of the Popocatepetl volcano (Zamora Rodríguez 1989).

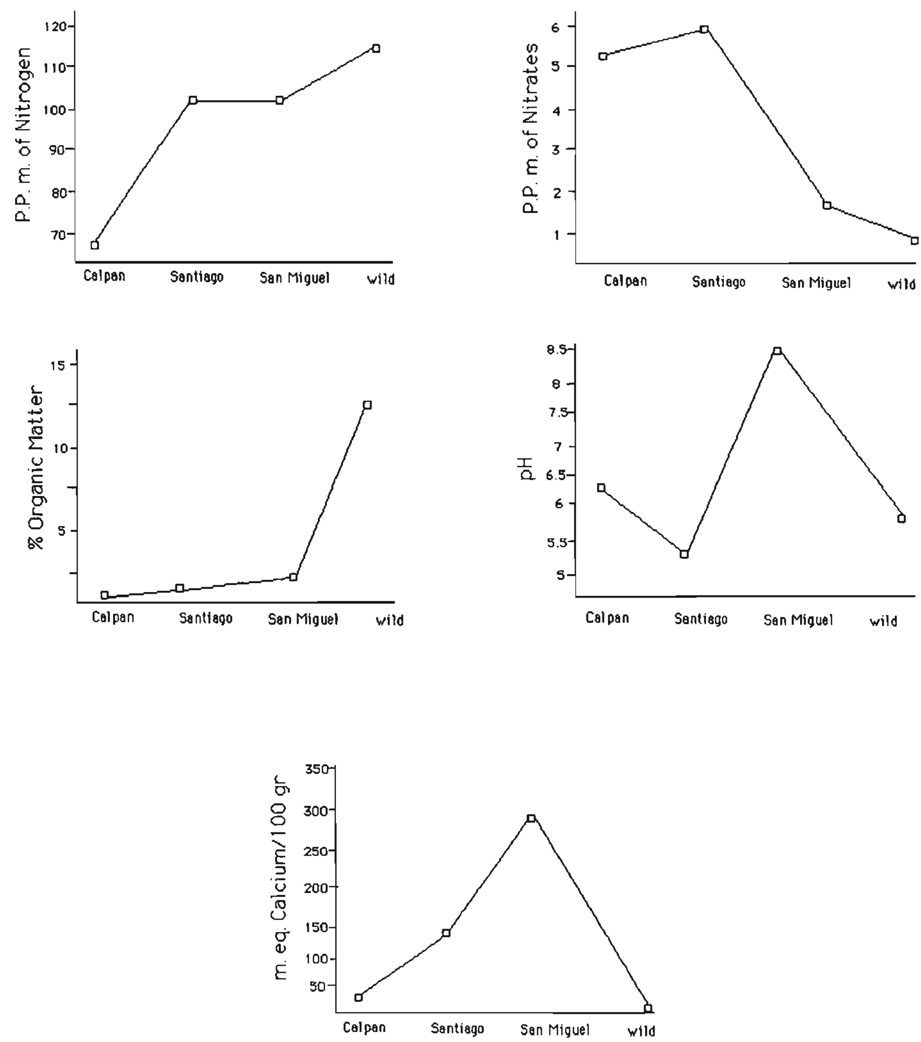

FIGURE 1.—Concentration of total nitrogen, soluble nitrates, organic matter, pH, and calcium in the soil of the studied sites.

Santiago Tepetlapa, Morelos (hereafter Santiago) is a small community with a long history of bean cultivation since pre-Columbian times (Souza 1990; Souza *et al.* 1992, 1994). Although the culture is Nahuatl, the language is being lost by the younger generations (Table 1). Rainfall at this site is fairly predictable and abundant in the summer (García 1988). Santiago has undergone significant socioeconomic changes in the last 10 years, because of its proximity to the village of Tepoztlán, an expensive resort for people from Mexico City. Many agricultural plots have been sold to outsiders to build country villas (V. Souza, personal observation). In Santiago the crops are cultivated mostly by men without the help of women. Women work mostly as housekeepers in the villas or at home. The soil in Santiago is acid and very rich in nitrates (Figure 1) due to the input of chemical fertilizers.

Tepoztlan, Morelos (hereafter, "wild site") is where we found wild populations of *P. vulgaris* and *P. coccineus* (Souza 1990; Souza *et al.* 1992, 1994). This is a very disturbed oak forest heavily grazed by cows and horses. It is located 3.7 km nw of the town of Tepoztlán and 7 km n of the town of Santiago. Rainfall in this area is fairly predictable and abundant in the summer. The soil at this site is the poorest of all in calcium but relatively rich in organic matter (Figure 1).

Ethnography.—We made eight visits to the communities of Calpan and San Miguel (four two-day visits to both sites). We interviewed women, taping the conversations, having first obtained permission from them to do so. Although a questionnaire was prepared, the interviews were made in an informal and casual manner that brought into conversation previously prepared questions. Subsequently, the questions and answers were analyzed in order to obtain general response patterns, to asses the way in which the women from these communities perceive their relationship with the crops they tend and with natural resources in their surroundings. The interviews took place mainly in homes, though a couple of times we approached the women while they were shopping, so that the persons accompanying them (usually their children or friends) participated as well. We interviewed 13 women in Calpan and 12 in San Miguel. In both places we sought to interview women of different ages.

Sampling procedures for the beans and R. etli.—Agricultural plots in San Miguel and Calpan were sampled during 1994 (Table 2). The plots were selected based on families that were willing to share their land and a portion of their bean crop so that we could do our research. The plants within each plot were selected using random numbers and a grid map. The approximate number of active nodules present in each plant was counted and ten active nodules were collected from each plant. A leaf sample of each sampled plant was collected and stored at -80° C, in order to assess the genetic structure of the plants for all plots. A soil sample from each plot was also taken. One hundred randomly selected seeds from each plot were measured and weighted and their color was scored. The productivity per plant was evaluated *in situ* by hand harvesting ten randomly selected plants per plot. The aerial part of the plant was stored in a large plastic bag, taking care to preserve all the loose leaves. The seeds from each plant were stored in a separate bag. In the laboratory the plants and seeds were dried in a stove at 350° C. The dry weight of the plants and seeds from each site were compared using a Student's *t*-test (Sokal and Rohlf 1981).

TABLE 2—Sampling scheme, characteristics of the beans, number of strains, and electrotypes (ETs) of *Rhizobium etli* obtained from each site.

Characteristics	Calpan, Puebla	Santiago, Morelos	San Miguel, Puebla
Sampled plots (year)	3 (1994)	1 (1987), 1 (1988)	3 (1994)
Sampled plants/plot	10	100 (1987)[a], 32 (1988)	10
Bean productivity (INEGI 1994)	high (2.5 ton/ha)	medium (1 ton/ha)	subsistence (ranges from1 kg to 0.5 ton/plot)
Average dry weight seed /plant (± s.d)	0.68 ± 0.13	n/a	0.890 ± 0.01
Average bean size (cm ± s.d)	1.23 ± 0.12	1.02 ± 0.10	1.42 ± 0.24
Average bean weight (gm ± s.d)	0.38 ± 0.09	0.32 ± 0.05	0.59 ± 0.31
Different colors of bean seeds	4	1	6
Bean variety	"amarillo" and "mantequilla", *P. vulgaris*, wild *P. coccineus*	"negro jamapa", *P. vulgaris*	landraces of *P. vulgaris*; cultivated *P. coccineus*
Average ot total nodules per plant (± s.d)	297 ± 27.61	105 ± 25.25	345 ± 48.32
Average active nodules /plant	34.50 ± 8.62	14.00 ± 5.21	48.50 ± 5.40
Average number of active nodules/1 gm root dry weight (± s.d.)	40.22 ± 4.93	not analyzed	98.74 ± 16.65
Number of strains isolated from 10 random plants	122	270	229
ET/stains	0.508	0.352	0.393

[a] From 99 plants only one nodule was extracted, while from one plant all the nodules (52) were extracted, see Souza *et al.* (1994).

Bacterial isolates.—The nodules were washed in 10% sodium hypochloride and rinsed twice with sterile water. Nodules were smashed in Petri dishes with Peptone Yeast Extract medium (PY, Souza 1990) and grown for two days at 30° C. One strain was isolated and grown again in a new Petri dish. This procedure was repeated twice to obtain a pure strain or clone. Each strain was kept at -80° C in UL (Glicerol-Peptone minimum media, Souza 1990; Souza *et al.* 1994). We isolated 351 strains for this analysis: 229 from San Miguel and 122 from Calpan, from three randomly selected plots at each site.

Electrophoresis procedures for beans.—We analyzed the genetic diversity of both species of beans in the sites we sampled using isoenzyme electrophoresis in cellulose acetate membranes (Hebert and Beaton 1993). Since most of the loci that have been studied in *P. vulgaris* are monomorphic (Escalante *et al.* 1994), we were able to analyze only three polymorphic loci: malic enzyme (ME, EC 1.1.1.40), isocitrate dehydrogenase (IDH, EC 1.1.1.42), and 6-phosphoglucose dehydrogenase (6-PGDH, EC 1.1.1.49). For *P. coccineus* four polymorphic loci were analyzed (ME, IDH, 6-PGDH, and malate dehydrogenase [MDH, EC 1.1.1.37] as an extra enzyme).

The genetic variation was estimated as the average expected heterozygosity in Hardy-Weinberg equilibrium, H (Hedrick 1983; Escalante *et al.* 1994) which ranges from zero, if there is no genetic variation, to a theoretical maximum of one, if there are an infinite number of alleles, each with the same allelic frequency.

Electrophoresis for bacteria.—Before each electrophoresis, the strains to be analyzed were grown in solid PY and two days later transferred to liquid PY (50 ml). After two days, the cultures were centrifuged at 6000 rpm during 5 min to obtain the cell pellet. The supernatant was eliminated and the pellet resuspended in 1 ml of Tris HCl pH8 buffer. Addition of 0.1 ml of lysozime (0.075 mg/ml) ensured lysis of the bacterial walls, and the resulting suspension was frozen twice at -80°C for 15 min. It was then centrifuged at 12,000 rpm during 5 min. The supernatant containing the protein lysate was distributed in three 1.5 ml plastic tubes and stored at -80°C. The strains from Morelos were analyzed using isoenzyme electrophoresis in starch as described by Selander *et al.* (1986) and Souza *et al.* (1994). For the strains from Puebla, electrophoresis was performed in membranes of acetate cellulose (Hebert and Beaton 1993). Seven polymorphic enzymes were used: isocitrate dehydrogenase (IDH, EC 1.1.1.42), peptidase (PEP, EC 3.4.11), phosphoglucomutase (PGM, EC 5.4.2.2), glucose 6-phosphate dehydrogenase (G-6PDH, EC 1.1.1.49), xanthine dehydrogenase (XDH, EC 1.1.1.204), malate dehydrogenase (MDH, EC 1.1.1.37), and malic enzyme (ME, EC 1.1.1.40).

Measurement of Bacterial Diversity.—From allele frequencies, the genetic diversity for an enzyme locus was estimated again as the expected virtual (since the bacteria are haploid) heterozygosity in Hardy-Weinberg equilibrium (H), and was estimated as $H = (1\text{-}Sx_i^2)[n/(n\text{-}1)]$, where x_i is the frequency of the i-th allele and n is the number of genotypes (or electrotypes, ET) (Selander *et al.* 1986; Souza *et al.* 1994). The average genetic diversities were calculated hierarchically considering the diversity of the rhizobia within each plant, within each plot, from each site, and the total diversity using the ETDIV program for bacterial population genetics (Whittham 1990).

Genetic diversity was also estimated by the number of electrophoretic morphs (electrotypes or ETs) divided by the number of strains analyzed. The higher value (n ETs / n strains = 1) is obtained when all the isolates are genetically different, and the lowest (1 / n strains) is obtained when all the isolates are identical. This estimate is obviously sensitive to the number of strains collected and the number of loci used in the analysis, but it reflects information on the degree of diversity and clonality of the population (Souza *et al.* 1994).

Genetic Differentiation of the Bacteria.—We used three modified indices related to the G_{st} index to estimate the genetic differentiation at three hierarchical levels (Souza *et al.* 1994):

1) plant level $G_{pp} = (H_{plots} - H_{plants}) / H_{plots}$
2) plot level $G_{ps} = (H_{sites} - H_{plots}) / H_{sites}$
3) site level $G_{st} = (H_{total} - H_{sites}) / H_{total}$

where H_{plants} is the average genetic diversity of *R. etli* within plants in a plot; H_{plots} is the average genetic diversity within plots; H_{sites} is the average genetic diversity within a community (Calpan or San Miguel) and H_{total} is the total genetic diversity of the sample in a given state (Puebla or Morelos). These indices range from zero, if there is no genetic differentiation at that level (this can happen if all units at that level have exactly the same alleles with the same frequencies), to one, if there is maximum genetic differentiation (meaning that the compared units share no alleles) (Souza *et al.* 1994).

RESULTS

Ethnographic Studies.—In this study, we held informal interviews with the people from both San Miguel and Calpan and observed their agricultural practices for a year. Important differences were observed in the way women from both communities grow their crops, manage their soils, and use the local vegetation (collection of medicinal and edible plants, fruit trees, and woody plants for fuel, as summarized in Table 3). The most interesting difference among women from these two communities is how they perceive their environment and select and store seeds, For example, more seed parameters (size, color, and general aspect) are used by the women in San Miguel than in Calpan.

TABLE 3. —Percentage of interviewed women that reported participating in each activity
(interviews: San Miguel, 12; Calpan, 13).

Activity	Calpan	San Miguel
Cultivate beans and maize in same plot	7.7%	91.7%
Weed by hand	53.3%	91.7%
Separate seed by size	76.9%	100.0%
Separate seed by color[a]	7.7%	33.3%
Separate corrugated or parasitized seed	30.8%	41.7%
Sow and harvest at new moon	0.0%	41.7%
Collect fuelwood	30.8	66.7%
Buy fuelwood	84.6%	33.3%
Collect edible and medicinal plants	46.2%	83.3%
Tend animals	46.2%	66.7%
Grow fruits/flowers in home gardens	100.0%	100.0%
Grow fruit trees and collect fruits	46.2%	0.0%
Weave palm	0.0%	16.7%
Are healers	0.0%	8.3%

[a] Each bean seed color represents a variety with different cooking and storage quality.

The observed differences could be due to the predominance of Nahuatl agricultural traditions in San Miguel and to the fact that in San Miguel there is an almost complete absence of men during many months each year due to migration to the U.S.A. The men return to their homes for the San Miguel festival (September) and for the winter (from November 1, to celebrate the dead, and from Christmas through early March for the preparation of the land). During these few months they discuss with the rest of the family future agricultural planning, seed selection, and the amount of land to be assigned to each cultivar. During the rest of the year, women see themselves as mere "helpers" of their absent husbands, even though women are in charge of the agricultural and commercial activities for much of the year. Calpan has a more "traditional" family structure in the sense that most men are present during the year, and women's activities are limited to that which the husband or father "allows them" to do (Table 3). In Calpan there has been a gradual loss of agricultural traditions, "Because working in the fields is not profitable any more, people are leaving; young people study but cannot find jobs, so they just stay around, doing nothing." At the same time, the seed stock of the community (germ plasm) has suffered pressure from the market, and local varieties have replaced commercial varieties which "sell better, although they do not taste as good."

Bean morphology and diversity.—Beans in San Miguel are larger and heavier than those from Calpan (see Table 2; t = 15.67, p<0.001; t = 18.72, p<0.001; respectively), and show a high diversity both in color (six colors in San Miguel; four colors in Calpan), size (variation coefficient = 17.21 in San Miguel; 10.21 in Calpan, Table 2), and weight (variation coefficient = 51.80 in San Miguel; 23.69 in Calpan, Table 2). The *criollo* varieties from San Miguel are also as rich in protein as those from Calpan (average = 3.26% total nitrogen in Calpan; 3.32% in San Miguel; analyses at the Instituto Nacional de Nutrici6n, Mexico City). Besides, the yield per plant (dry weight of the seeds/aerial dry weight of the plant) is significantly larger in San Miguel than in Calpan (Table 2; t = 6.9, p<0.001).

Cultivated and wild *P. vulgaris* have very low levels of genetic variation (Figure 2; *H* ranges from 0.016 to 0.025), with slightly higher levels for the wild *P. vulgaris* and the cultivated population from San Miguel (*H* = 0.025 and 0.023, respectively). Low levels of genetic variation for cultivated *P. vulgaris* have been described previously by Escalante *et al.* (1994). The slightly higher genetic diversity in San Miguel may be due to a more diverse stock of local seeds. The germplasm in San Miguel is actively and carefully maintained by each family, and the criteria to harvest, store, and select seeds is more complex than in Calpan (Table 3), where the seed supply is changing constantly due to the input of government agencies.

Phaseolus coccineus populations have substantially higher levels of genetic diversity than the common bean (Figure 2; range of *H* is 0.24-0.368). The cultivated population of *P. coccineus* in San Miguel has lower variation levels than *P. coccineus* from other localities in central Mexico (*H* = 0.24), which may be due to the fact that the weather and soil in San Miguel are not part of the normal ecological range of this species, which occurs naturally in temperate oak forest with cooler climate and higher humidity (Escalante *et al.* 1994).

Genetic Structure of Rhizobium etli.—Genetic diversity of rhizobia and beans: The *H* values and the ET/strains indices and their relationships with the variation levels of the host plants are shown in Figure 2. In terms of *H*, rhizobia associated with wild *P. vulgaris* and wild *P. coccineus* have lower levels of genetic variation (*H* range is 0.118 to 0.335), while the rhizobia associated with both cultivated *P. vulgaris* and *P. coccineus* have higher levels of genetic variability, with *H*, ranging from 0.407 to 0.542 (Figure 2 and Table 4). The ETs/strains index indicates, again, that the wild *P. vulgaris* associated rhizobia have the lowest levels of genetic variation. while the second lowest are the cultivated *P. vulgaris* population. The highest levels of genetic variation occur in the rhizobia associated with *P. coccineus*, regardless of being wild or cultivated. When we compare the levels of genetic variation of the host plants with their associated bacteria, in *P. vulgaris* there is a negative correlation between plant and bacterial genetic variation (Figure 2). This is explained, at least in part, by the ecological dominance patterns discussed below.

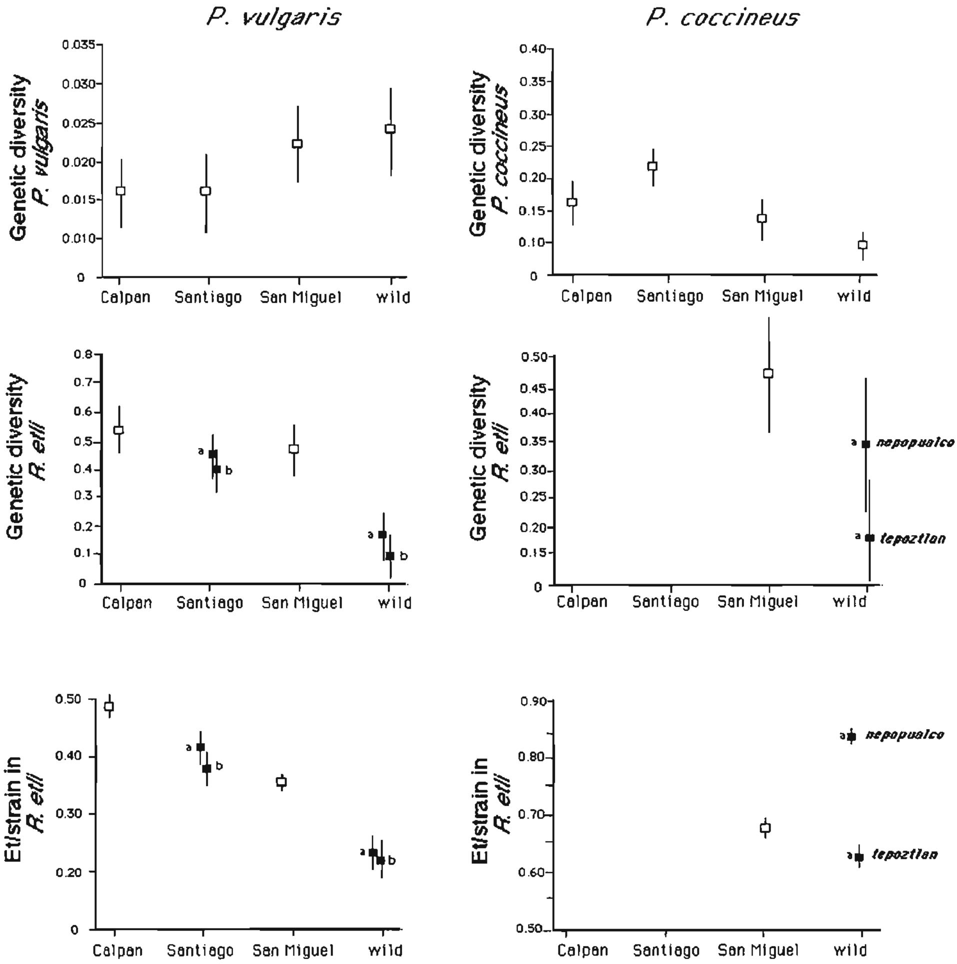

FIGURE 2.—Genetic diversity of beans and associated rhizobia in four sites in Mexico. The first column shows the genetic diversity of *Phaseolus vulgaris* and the genetic variation of their associated *Rhizobium etli*; the second column shows the genetic diversity of *P. coccineus* and associated *R. etli* (see text). The rhizobia associated with *P. coccineus* in Calpan, Puebla and in Santiago, Morelos are not represented in the figure due to the small sample size. Open squares show the data of 1994; filled squares show the data from Souza *et al.* 1994; [a] is for the 1987 studies; and [b] is for the 1988 studies. The bars represent one standard error.

TABLE 4.—Relative genetic differentiation in *Rhizobium etli* in Puebla and Morelos.

Site	N strains[a]	*H R. etli*[b]	G_{pp}[c]	G_{ps}[d]
Calpan, Puebla	123	0.545		0.101
plot 1	54	0.463	0.052	
plot 2	33	0.466	0.014	
plot 3	36	0.540	0.009	
Santiago, Morelos[e] (1988)	190	0.407	0.58	
San Miguel, Puebla	229	0.508		0.142
plot 10	95	0.498	0.379	
plot 11	74	0.440	0.555	
plot 12	60	0.371	0.482	
Wild *P. vulgaris*, Morelos[e]	33	0.118	0.53	
Total: Puebla	351	0.579	G_{st}[f] = 0.09	
Total: Morelos[e]	223	0.487	G_{st}[f] = 0.55	

[a] Total number of isolated and identified strains

[b] *H R. etli*: A measure of the genetic variation in *Rhizobium*, the virtual expected heterozygosity (Souza *et al.* 1994).

[c] Relative genetic differentiation among plants within a plot ($G_{pp} = H_{plot} \cdot H_{plant} / H_{plot}$).

[d] Relative genetic differentiation among plots within a site ($G_{ps} = H_{site} \cdot H_{plot} / H_{site}$)

[e] Based on data from Souza et al. 1994: G_{ps} not obtained for Morelos samples.

[f] Relative genetic differentiation among sites within each state ($G_{st} = H_{total} \cdot H_{site} / H_{total}$)

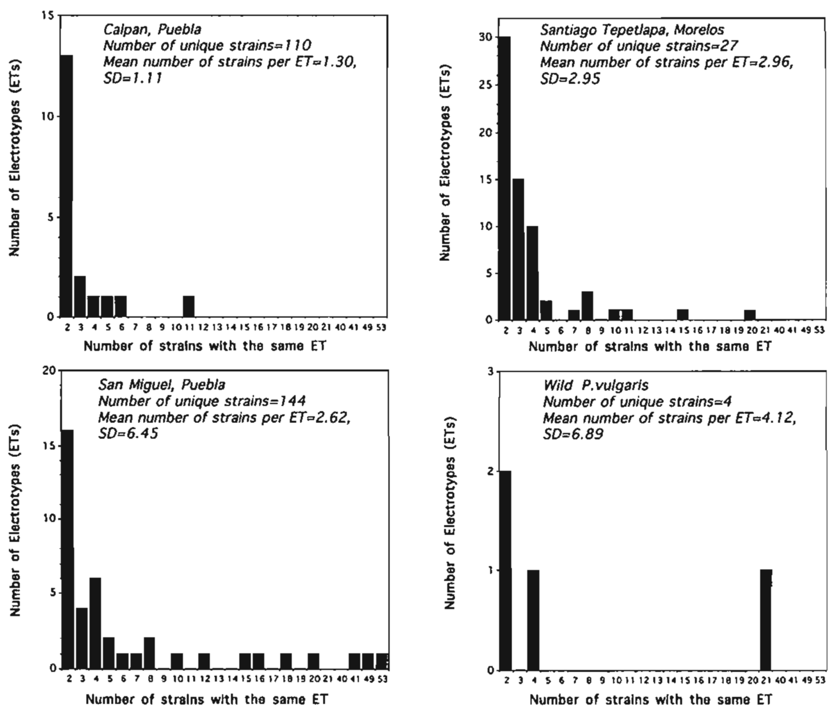

FIGURE 3.—Distribution of the frequencies of strains with the same genotype (ET) in the study sites.

Distribution of the strains and degree of dominance: In Figure 3 we show the distribution of strains with the same ET (electrotype) for four contrasting populations of rhizobia. We detect a gradient of ecological dominance, which appears to depend on the degree of agricultural management. In Calpan, the most technified site, the community of *R. etli* presents the lowest ecological dominance, with many ETs represented by just one or few strains in each, so the average number of strains per ET is 1.30. Both communities with simpler agricultural technology, Santiago Tepetlapa and San Miguel, have intermediate dominance levels, with most ETs being unique or with few strains, but some ETs are represented by several strains, and the average number of strains per ET is 2.96 and 2.62, respectively. The wild *P. vulgaris* site is the extreme of the gradient, with a strong ecological dominance, as most of the collected strains belong to a single ET, and, in consequence, the average number of strains per ET is the highest (4.12)..

Genetic differentiation at different hierarchical levels: The genetic levels of differentiation are described by the G_{st} analogs at the different levels, shown in Table 4. In Calpan, the G_{pp} indicates there is very little genetic differentiation of the bacterial isolates among plants in a given plot (G_{pp} range 0.009-0.052), and thus we found in each plant most of the rhizobia variation described for the entire plot. In contrast, in San Miguel the G_{pp} was substantially larger, ranging from 0.379 to 0.555, indicating a large degree of genetic differentiation of the bacterial isolates among plants. This means that each plant had a lower proportion of the rhizobia genetic variation found in the whole plot. This pattern was also found in the cultivated rhizobia from Santiago, (G_{pp} = 0.58) and in the wild *P. vulgaris* (G_{pp} = 0.53) where most of the variation is found between plants. At a higher level, in both communities in Puebla we found that each plot had most of the genetic variation present in that site (Calpan G_{ps} = 0.101, San Miguel G_{ps} = 0.142), meaning that there was little bacterial genetic differentiation among the plots in a given site. At the site level within each state, the G_{st} indicates that each site in Puebla represented most of the genetic variation, and very little genetic differentiation was found between sites (G_{st} = 0.09), while the sites sampled in 1987 in Morelos were quite different (G_{st} = 0.55). On the other hand, we have not found a single shared strain between the two sites in Puebla, although *P. coccineus* and *P. vulgaris* in San Miguel shared 5 ETs. In Morelos, very few strains were shared among neighboring sites (Souza *et al.* 1994). This may be due in part to the lack of mobility of this bacteria, but also to a lack of adaptation to new environments.

DISCUSSION

The main findings of our research are: 1) San Miguel is a Nahuatl community that has maintained its agricultural tradition and its rich and diverse bean germ plasm for generations. In recent years, women have played an important role in preserving this tradition. In Calpan, on the other hand, the seed stock of the community has suffered increasing pressure from the market; local varieties of beans have been replaced by commercial varieties that are smaller and less diverse than the beans in San Miguel. 2) The genetic diversity of *R. etli* associated with cultivated beans, both *P. vulgaris* and *P. coccineus*, is high in all the communities studied, while it is lower for the *Rhizobium* associated with wild beans. 3) The population structure of *R. etli* is different in all the populations studied. The most fertile and intensively managed plots are similar, while the least managed plots resemble the wild site of *P. vulgaris*; the genetic structure of *Rhizobium* seems to be associated with agricultural practices, bean genetic diversity, and soil conditions.

The people, the beans and the environment.—One of the objectives of this research was to assess the role women play in these communities as managers of their natural resources and germ plasm, and therefore, to evaluate their participation in the conservation and management of the genetic diversity of beans and the nitrogen-fixing bacteria *Rhizobium etli*.

Women in San Miguel appreciate the heterogeneity in their crops, as was expressed by a woman in an interview: "*Me gusta que mi canasta esté pinta*" ("I like a mottled basket."), referring to the diversity of color and size of her corn and bean seeds (for instance, tortillas are brownish in San Miguel due to a mixture of grains of different colors). In the interviews we observed that women in San Miguel take more variables into account when they select their seeds for the next cycle. This selection for heterogeneity is confirmed by the morphological and genetic analysis of the beans, where the maintenance of a mixture of genotypes is evident. The beans in San Miguel are also larger and heavier than the commercial seeds from Calpan. A higher diversity of beans, such as we found in San Miguel, has been observed elsewhere in areas where local landraces are maintained (Brush 1986; Altieri and Merrick 1987; Martin and Adams 1987). While genetic diversity is directly related to ancient agricultural traditions and may be explained as a response to a complex and competitive ecological environment, it may also associated with low productivity (Jennings and Cock 1978). This is only partially true for San Miguel, where bean productivity per hectare is lower than in Calpan and is, in fact, one of the lowest in the state of Puebla (INEGI 1994), but where yield per plant is significantly higher. This paradox is explained by the fact that people in San Miguel cultivate beans varieties that grow as vines. These plants can grow so heavy that they can crush the maize plants that provide their support. The women in San Miguel choose only a certain number of maize plants to support their beans and do not have more than a few hundred plants per hectare. In contrast, bean varieties cultivated in Calpan are free-standing and can be grown as a monoculture, with densities as high as 25,000 plants per hectare.

The bean plants in San Miguel also may be better symbionts with rhizobia than the beans in Calpan, as indicated by the number of active nodules per plant (red nodules evidence active nitrogen fixation) and the dry weight of nodules/gm of dry root (Table 2). In San Miguel the average number of nodules per plant was 229, while in Calpan it was only 122. However, these results need to be replicated in the greenhouse by controlled inoculation of different seeds.

In addition to the rich bean germ plasm, the botanical research indicates that in spite of the region´s semi-arid climate and eroded soils, San Miguel has a higher plant diversity within its fields than Calpan, which is explained in part by the Nahuatl agricultural practices that promote the growth of useful weedy species, including medicinal herbs used by healers in the community. While most women in San Miguel collect plants and fuel wood from their plots and immediate surroundings, women in Calpan buy fuel and medicinal plants at the market; when they do collect plants, they do not gather them in the agricultural plots.

Agricultural techniques.—Agricultural traditions differ in the two communities. Plots in San Miguel are seldom hand-weeded and fertilized. This may lead to a certain degree of nutrient competition among the various plants growing together in the plots by reducing the amount of resources available to the bean plants. Bean plants from Calpan, where fields are managed intensively and fertilized, obtain more nutrients. Peasants in San Miguel, however, obtain other benefits from the presence of a tolerable level of weeds in their fields (Bye 1981). In San Miguel, 11-21 species of weeds are found in the plots and most of these are used for medicinal and culinary purposes, while in Calpan only six-eight species are found in association with the beans. Weed communities may also enhance biological insect pest control (Altieri *et al.* 1977), organic matter accumulation, and soil conservation (Chacon and Gliessman 1982). The higher number and weight of the active nodules in plants from San Miguel may be due to both the differences in beans varieties and the low levels of agrochemicals, since large amounts of ammonia and nitrates in the soil inhibit the nodulation process (Long 1989).

Even though the common beans in San Miguel are much more diverse morphologically than the beans in Calpan, *P. vulgaris* genetic structure is similar in the four sampled sites. These results suggest that the morphological diversity may be due to the expression of a few genes that are not scored in the multiloci electrophoresis. In that case, even if the mating system of the species reduces heterozygosity (Escalante *et al.* 1994), the selection criteria for seeds in San Miguel may be favoring morphological diversity by selecting diverse bean lines that coexist in a cultivar.

The genetic erosion of crops is common all over the world at the sites of domestication. The replacement of local landraces by commercial varieties usually implies the loss of all or most of the old cultivars and their genetic diversity. Although yields may rise through this substitution, an increase in management costs and a reliance on purchased inputs, like fertilizers and seeds, is common (Brush 1986; Altieri and Merrick 1987).

Rhizobium *genetic structures and diversity.*—In San Miguel, the community with minimal tilling, the ecological dominance is high, as three ETs (electrotypes) represent more than 50% of the total population. Nodulation is also high, but environmental pressures appear to reduce the genetic diversity (measured as the ratio of ETs to strains), and increase the differentiation from plant to plant within a plot. The abundance of some well-adapted ETs (i.e., ecological dominance), suggests both adaptation to the local soil conditions and adaptation to the local bean varieties (Souza 1990). This hypothesis is reinforced by the number of nodules per plant and the ratio of strains per ET. In contrast, in Calpan the soil conditions are good and the technologically modified agriculture may increase the movement of strains within a plot, and in consequence reduce local adaptation of the strains. In this site, the ecological dominance is the lowest, and both the ratio of ETs to strains and the genetic diversity are high, but nodulation efficiency is lower than in San Miguel. In Santiago the ecological dominance and low nodulation efficiency may be explained by the low calcium concentration (Lodeiro *et al.* 1995). Furthermore, in Santiago there appears to be a low degree of migration of strains from other sites (Souza *et al.* 1995), which limits the genetic diversity within each site and increases the genetic differentiation. In the wild site of Tepoztlan, the ecological dominance was the highest, as one ET represented most of the strains. This site had also the lowest genetic diversity and high levels of genetic differentiation (Souza *et al.* 1994).

Agricultural practices may have indirectly changed the genetic structure of the nitrogen fixing bacteria *R. etli*. The bacteria associated with cultivated plants have higher levels of genetic variation than those associated with wild plants. This result may seem paradoxical, as it is well known that most domesticated organisms have lower levels of genetic variation than their wild relatives (Doebley 1989). Greater genetic diversity in *Rhizobium* may be due solely to agricultural practices: there may be more bacteria in the soil in cultivated plots than in the wild sites, and thus the plants may be able to sample from a larger pool of genotypes. On the other hand, the greater genetic diversity may reflect changes in the plant specificity due to domestication. If this is the case, the roots of the cultivated plants can be colonized by a genetically wider pool of bacteria than the wild beans. We suspect that a change in specificity is a more likely explanation (see also Souza *et al.* 1994). Future experiments on the specificity of both beans and rhizobia will test this hypothesis. These results suggest that the genetic structure of *Rhizobium* depends not only on the number of different strains found in a site, but also on the biology of the host plant and on the agronomic practices of each community.

Ethnomicrobiology, an emerging field.—The process of plant domestication, as well as introduction of crops to novel environments, may have an impact on both the introduced and the native rhizobia (Souza 1990). The extent of this effect has not been evaluated (Martínez-Romero and Caballero Mellado 1996). In this study we observed that rhizobia performance is much better in San Miguel than in Calpan. The efficiency of the interaction between *R. etli* and the local races of beans in San Miguel may be due to seed selection, crop management, and the adaptation of the *R. etli* and the local beans to alkaline soils and unpredictable rains. The introduction of nitrogen by way of fertilizers and the use of novel and homogeneous bean varieties may be changing the genetic structure of the native rhizobia and their interaction with beans. Such changes could be affecting other microbes associated with crops. The direct and indirect effects of traditional human activities on the microbiota are overlooked, yet potentially important aspects of ethnobiology. We suggest that the interdisciplinary study of the biological, ecological, and cultural aspects of the interactions between microbes and humans constitutes the field of ethnomicrobiology.

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