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Dispersal of the cotton boll weevil (Coleoptera: Curculionidae) in South America: evidence of RAPD analysis

M. A. Scataglini[1], V. A. Confalonieri[1] & A. A. Lanteri[2]
[1]*Lab. de Genética, Dto. de Cs. Biol. FCE y N, UBA, 4° piso, Pabellón II, Ciudad Universitaria, 1428 Buenos Aires, Argentina (E-mail: bibilu@bg.fcen.uba.ar);* [2]*Dep. Científico de Entomología, Fac. de Cs. Nat. y Museo, UNLP, Paseo del Bosque s/no, 1900 La Plata, Argentina (E-mail: alanteri@museo.fcnym.unlp.edu.ar)*

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Abstract

RAPD technique provides useful information on the geographic origin and dispersal of the boll weevil *Anthonomus grandis* in South America. Nine populations from Argentina, Brazil, Paraguay, Mexico and USA were analyzed. Weevils were captured on native plants (Misiones province, Argentina) and on cotton cultures, except the sample from the United States (USDA laboratory-reared colony). A sample of the 'Peruvian square weevil', *A. vestitus*, from Ecuador, was included in the analysis in order to compare interspecific variation. The four primers used in the analysis revealed 41 'anonymous loci'. The neighbor-joining tree based on Nei's distances and values of Nm (migrants per generation), indicate that genetic similarity between samples from Tecomán (Mexico) and Puerto Iguazú (Argentina), is higher than among remaining South American populations. This result supports an hypothesis of natural occurrence of the boll weevil in South America, prior to extensive cotton cultivation. Population outbreaks of the species would be associated with increase of agricultural lands.

Introduction

The boll weevil *Anthonomus grandis* Boheman, is the most harmful insect pest of cotton in the Americas (Lanteri, 1999). Since its first report damaging cotton in the United States (1894) there have been considerable efforts to address the question of its place of origin, native hosts and pathways of dispersal (Townsend, 1895; Fryxell & Lukefahr, 1967; Burke & Cate, 1979; Burke et al., 1986). Most authors proposed that the boll weevil was indigenous to tropical lowlands of Meso-America (southern and southeastern Mexico) and reached its present distribution by extending its range in both prehistoric and historic times.

The hypothesis of Meso-American origin of the boll weevil is based on evidence from its geographic variation, relationships with other Mexican and Central American Anthonomines, and host plants (Burke et al., 1986). These authors, suggested that the northward expansion of *A. grandis*, to northwestern Mexico and southern Arizona, as well as northeastern Mexico, took place before or during Pleistocene times, and followed a host shift from *Hampea* to wild species of *Gossypium* and other Gossypieae. On the contrary, the rapid dispersal of the boll weevil across the Cotton Belt of the southeastern United States occurred in historical times, and has been possible only through the availability of cultivated cotton as a host (Burke et al., 1986).

The migration of *A. grandis* into West Indies and South America is poorly known. Burke et al. (1986) proposed that its occurrence has apparently taken place within historical times and weevils may have arrived by separate importations from different areas. The species was first reported from Haiti (Audant & Occenad, 1937) as a result of an introduction from somewhere along the Gulf Coast of the United States. It occurred in Venezuela in 1949 and in Colombia in 1951. More recently, it was first reported in Brazil (Sobrinho & Lukefahr, 1983), and subsequently

in Paraguay (1991), Argentina (1993), and Bolivia (1997) (Lanteri, Confalonieri & Scataglini, 1998, 1999).

Molecular techniques, including allozyme data, DNA restriction fragment length polymorphisms (RFLP), mitochondrial DNA sequence analysis, and randomly amplified polymorphic DNA analysis (RAPD), can contribute to elucidate geographical origin and routes of dispersal of insect populations (Terranova, Jones & Bartlett, 1991; Roehrdanz & North, 1992; Confalonieri et al., 1998). The RAPD technique (Welsh & Mc Clelland, 1990; Williams et al., 1990) is becoming widely used with a variety of applications (Hadrys, Balick & Schierwater 1992; Landry, Dextraze & Boivin, 1993; Aljanabi et al., 1998; Confalonieri, Remis & Scataglini, 2000) and it is shown to be a powerful technique for reconstructing the phylogenetic history of insect pest populations, especially if they have diverged recently (Lenney Williams et al., 1994; Taberner, Dopazo & Castanera, 1997).

The main goal of the present contribution is to provide insights into the geographical origin and dispersal of South American populations of the boll weevil, through the analysis of their genetic similarities and comparisons with potential source populations, using RAPD technique. Genetic differences between them will depend on several factors, including gene flow, time since separation, genetic variation in the source population, number of founding events and selection pressures (Baker & Stebbins, 1965; Hartl, 1980; Templeton et al., 1990).

Materials and methods

Population sampling

Nine populations of the boll weevil *A. grandis* from Argentina, Brazil, Paraguay, Mexico and USA were analyzed. Moreover, a sample of the 'Peruvian square weevil', *A. vestitus* Boheman (Burke & Cross, 1966), from San Clemente, Manabí province, Ecuador, was included in the analysis in order to compare interspecific variation. The locations of collection sites and sample sizes are shown in Figure 1 and Table 1.

Some weevils were captured on native plants (populations from Misiones province, Argentina) and the remaining on cotton cultures, except the sample from Mississippi (United States), that corresponds to a USDA laboratory-reared boll weevil colony. This colony was begun many years ago from field-collected boll weevils belonging to this locality, and has been infused with wild stocks periodically. The collections of insects in the field were performed using traps containing 'Glandure' pheromone as described in Manessi (1997), and preserved in ethanol 100%.

DNA isolation and amplification

DNA was isolated from insects according to the method applied by Reiss, Schwert & Ashworth (1995). Four decamer primers (Promega) were used: AO1: 5′-CCCAAGGTCC; AO9: 5′-CTAATGCCGT; BO3: 5′-ACTTCGACAA; BO6: 5′GTGACATGCC.

The amplification conditions were based on Williams et al. (1990). The reaction was performed in 2 mM Tris–HCl (pH = 8); 10 mM KCl, 0.01 mM EDTA, 0.1 mM DTT, 5% glycerol, 3 mM MgCl2, 100 μM of each dNTP, 150 ng of each primer, 50–100 ng of total genomic DNA and 2.5 unit of *Taq* polymerase (Promega) in final volume of 50 μl. DNA amplification was done in thermal cycler (Techne). The first period of denaturation was 94°C for 6 min; then 40 cycles of denaturation, annealing and extension which consisted of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min. Finally, one extension cycle of 72°C for 5 min. Two negative controls (absence of template and Taq) were performed for each series of amplifications.

The amplification products were separated on 1.5% agarose gels with TAE buffer containing 0.5 mg/ml of ethidium bromide. Electrophoresis was carried out at 5 V/cm for 3 h.

Data scoring and analysis

Two replicate runs were made to determine the reproducibility of RAPD bands. Only reproducible bands were taken into account to generate the matrix dataset. In instances where two or more phenotypic classes were classified as fragment intensity differences, the fragment was counted only if one of the classes was virtual absence, and the intensity variants were summed into a single 'presence' class. These bands were considered identical in two individuals if they have similar mobility. Amplified fragments per individual were recorded as present (1) or absent (0). Since we analyzed intraspecific variation, reproducible products that comigrated were assumed to represent homologous loci, each one with two alleles with dominance relationship. We considered that percentage of codominant loci is low enough

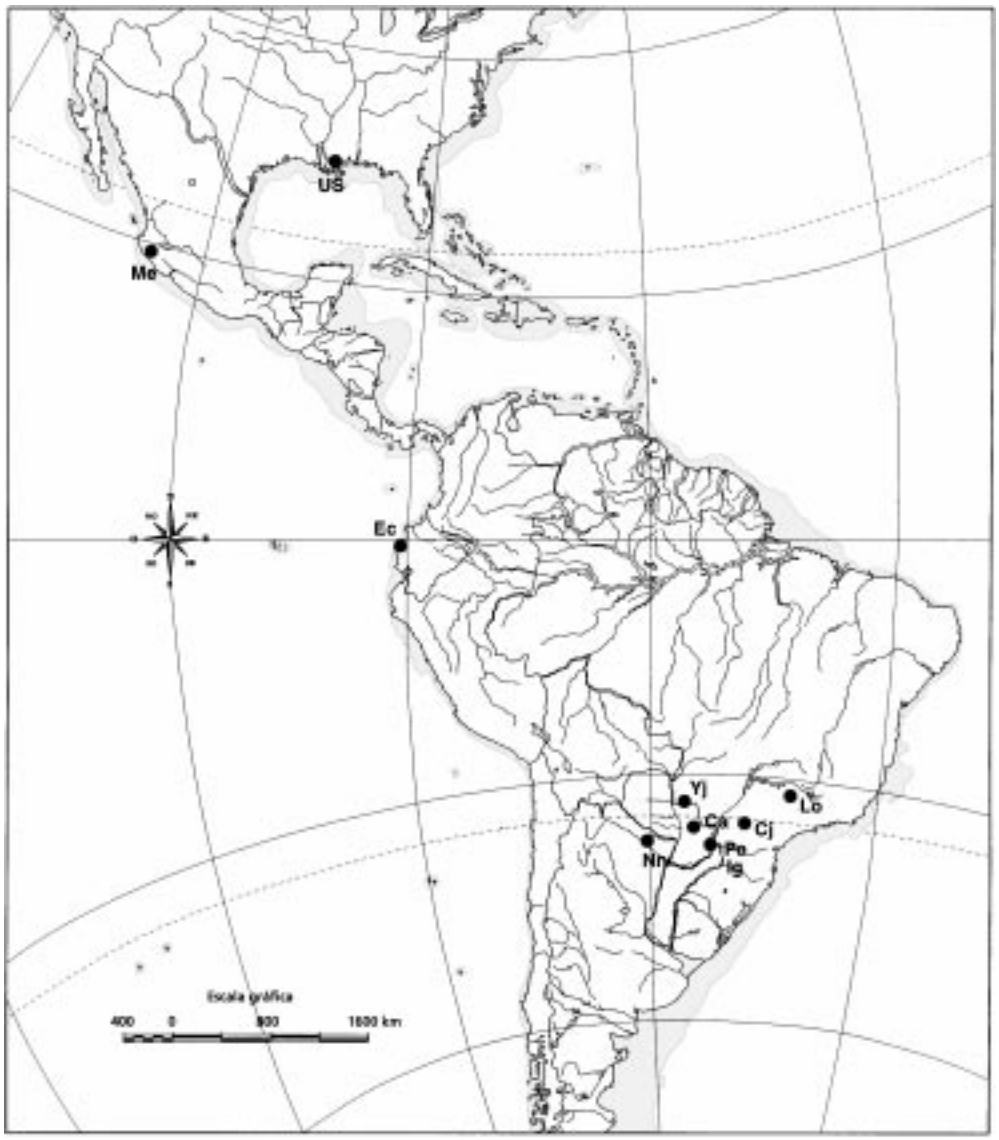

Figure 1. Geographic location of *A. grandis* samples studied. The only sample from Ecuador corresponds to *A. vestitus*. Acronyms according to Table 1.

to affect estimations of allele frequencies, and possible errors are compensated by the number of loci analyzed.

Allele frequencies and genetic variability measures were estimated using the computer programs RAPDBIOS 2.0 (Black, 1996) and BIOSYS-1 1.7 (Swofford & Selander, 1981). These population parameters were calculated assuming that genotypes are in Hardy-Weinberg equilibrium. Lynch and Milligan (1994) correction was applied when estimating allelic frequencies. Nei's distances (1972) among populations were computed with the RAPDDIST program (Black, 1996). The treatment of these data was carried out using the neighbor-joining method with the computer program NEIGHBOR of the PHYLIP 3.5 C package (Felsenstein, 1993). Reliability of the tree was tested using bootstrap analysis with 1000 replicates. Fst estimates and effective migration rates (Nm) were calculated by the Weir and Cockerham (1984) method of RAPDFST computer program (Black, 1996), which includes a correction for small and unequal sample sizes. Matrices of genetic and geographic distances were compared by mean of a Mantel test (Mantel, 1967).

Table 1. Information of samples of *Anthonomus grandis* studied (the only sample from Ecuador corresponds to *A. vestitus*)

Sample	Acronym	Geographic location	Number of individuals analyzed	Data of collection
Puerto Iguazú	Ig	Misiones Prov. Argentina	10	Natural population collected on native plants
Puerto Península	Pe	Misiones Prov. Argentina	8	Natural population collected on native plants
Laguna Naick Neck	Nn	Formosa Prov. Argentina	9	Natural population collected on cotton culture
Caacupé	Ca	Cordillera Prov. Paraguay	11	Natural population collected on cotton culture
Yjhoví	Yj	Canindeyú Prov. Paraguay	11	Natural population collected on cotton culture
Carajá	Cj	Paraná St. Brazil	8	Natural population collected on cotton culture
Londrina	Lo	Paraná St. Brazil	9	Natural population collected on cotton culture
Tecomán	Me	Colima St. Mexico	9	Natural population collected on cotton culture
Mississippi	US	Mississippi St. USA	11	Laboratory reared sample
San Clemente	Ec	Manabí Prov. Ecuador	10	Natural population collected on cotton culture

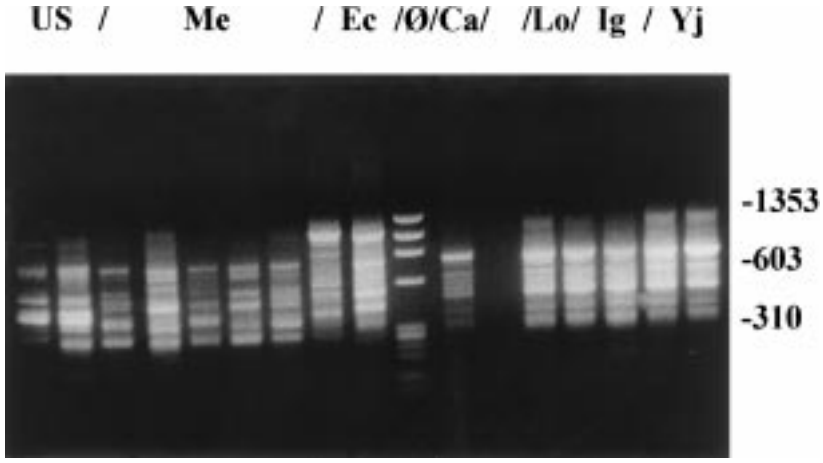

Figure 2. RAPD fragments amplified by AO1 primer, showing variability display in populations studied. ϕ = ladder. Acronyms according to Table 1. Fragment sizes are indicated on the right side of figure.

Results

The primers chosen for the analysis revealed 41 reproducible products which were assigned to 'anonymous loci', of 200–1500 bp (Figure 2). Eleven 'loci' were amplified with primer A01, 14 with primer A09, nine with primer B03 and seven, with primer B06. Amplification products A09-11, 12, 13 and 14, could be considered as diagnostic for *A. vestitus*, because they are fixed in this sample, and absent or politipic in *A. grandis* populations; on the contrary, B03-6, is fixed in *A. grandis* and polymorphic in *A. vestitus* (Table 2). Other loci, are useful to differentiate South American populations of *A. grandis* from samples from Mexico and/or USA, and to interprete their possible relationships (e.g. A01-04, 08; B06-03, 04, 05; A09-05) (Table 2). Remaining loci show variable frequencies in each population.

The mean heterozygosity (unbiased estimate of Nei, 1978) and the percentage of polymorphic loci estimated from allele frequencies, revealed a considerable amount of population variation, even in the laboratory reared sample, which displayed one of the highest mean heterozygosity per locus ($0.0229 + / - 0.035$). Moreover, samples from Puerto Iguazú and Tecomán, showed the highest percentage of polymorphic loci (60.98%) (Table 3).

Nei's distances (1972) using Lynch and Milligan's (1994) correction for RAPD loci between all pairs of samples, are described in Table 4. This matrix was analyzed using neighbor-joining algorithm. The tree obtained (Figure 3) points the sample of *A. vestitus* as

Table 2. Population frequencies of the RAPD locus dominant allele corresponding to each band (estimated following Lynch & Milligan, 1994) from primers AO1, AO9, BO3 and BO6. Numbers of bands were assigned in decreasing order of size. Acronyms of populations according to Table 1

Population primer	Ig	Pe	Nn	Ca	Yj	Cj	Lo	Me	US	Ec
AO1-01	0.357	0.429	0.063	0.357	1	0.167	0.100	0.250	0.214	0.500
AO1-02	0.071	0.071	0.063	0	0.214	0	0.100	0.200	0.429	0.375
AO1-03	0.214	0.125	0.125	0.550	1	0.214	0.167	0	0.357	0.625
AO1-04	1	1	1	1	1	1	1	1	0.429	0.500
AO1-05	0.357	1	0.625	1	1	0.500	1	1	0.429	0.375
AO1-06	0.357	1	1	1	1	0.625	1	0.200	0.143	0.250
AO1-07	0.571	1	1	1	1	1	1	0.500	0.357	0.583
AO1-08	1	1	1	1	1	1	1	0.500	1	1
AO1-09	0.429	1	1	0.650	1	1	1	0.500	0.214	0.375
AO1-10	0.143	0.071	0.188	0.300	0	0.125	0	0.250	0.571	0.250
AO1-11	0.071	0	0	0.050	0	0.188	0	0	1	0.063
AO9-01	0.056	0.214	0.500	1	1	0.500	1	0.063	0	0
AO9-02	0.056	1	1	1	1	1	1	0.063	0	0
AO9-03	0.333	0.571	1	0.625	0.611	1	1	0.188	0.056	0.100
AO9-04	0	0	0.111	0.056	0	0	0	0	0.222	1
AO9-05	1	1	1	1	1	1	1	1	0.611	0.300
AO9-06	0.063	0.188	0.389	0.111	0.222	0.063	0	0.125	0.222	0
AO9-07	1	1	0.625	1	1	1	1	0.375	1	0.050
AO9-08	0.111	0.500	0.625	0.063	0.188	1	0.500	0.500	0.389	0
AO9-09	1	0.500	0.625	0.583	0.417	0.583	0.333	0.125	0.389	0
AO9-10	0	0.571	0.333	0.500	1	0.125	0.188	0.111	0	0.150
AO9-11	0.050	0	0	0	0	0	0	0.222	0.045	1
AO9-12	0	0	0.111	0	0	0	0	0	0.045	1
AO9-13	0	0.063	0	0	0	0	0	0	0.045	1
AO9-14	0.056	0	0	0	0	0	0	0.143	0	1
BO3-01	0.150	0.125	0	0.167	0	0.500	0.500	0	0.045	0.611
BO3-02	0.100	0.625	0.429	0.167	0.375	1	1	0.143	0.045	1
BO3-03	0.350	1	1	0.333	0.571	1	0.429	0	0.091	1
BO3-04	0.150	1	0.167	0.056	0	1	1	0.167	0.455	1
BO3-05	0.150	0.188	0.611	0.333	0.500	1	1	0.417	0.091	0.188
BO3-06	1	1	1	1	1	1	1	1	1	0.650
BO3-07	0.063	0.188	1	0.167	0.125	1	1	1	0.636	0.611
BO3-08	1	1	1	0.500	0.625	1	1	0.611	0.364	0.650
BO3-09	0.625	1	1	0.111	0	1	1	0.417	0	0.650
BO6-01	0.056	0.188	0	1	0.450	0	0	0	1	0.167
BO6-02	0	0	0	0	0	0	0	0	1	0.063
BO6-03	1	1	1	1	1	1	1	0.500	0.625	1
BO6-04	1	1	1	1	1	1	1	1	0	1
BO6-05	0.100	0.375	0.571	0.100	0.056	0.500	0.429	0.222	1	1
BO6-06	1	1	0.214	0.611	0.625	1	0.571	1	0.056	0.056
BO6-07	1	1	1	1	1	1	0.429	0.625	0	1

Table 3. Estimates of genetic variability. H = Mean heterozygosity per RAPD locus (unbiased criterion). Pl = Percentage of polymorphic loci (0.95 criterion). Acronyms according to Table 1. Ec = *A. vestitus* sample from Ecuador

	Ig	Pe	Nn	Ca	Yj	Cj	Lo	Me	US	Ec
H	0.173± 0.030	0.151± 0.032	0.169± 0.034	0.181± 0.034	0.134± 0.033	0.118± 0.032	0.117± 0.033	0.238± 0.035	0.229± 0.035	0.230± 0.036
Pl	60.98	41.46	43.90	51.22	31.71	29.27	26.83	60.98	53.66	58.54

Table 4. Above the diagonal: Nei's genetic distances (1972) obtained from RAPD allele frequencies. Below the diagonal: Fst and effective migration rates (between brackets) obtained by Weir & Cockerham (1984) method. Acronyms correspond to those given in Table 1. Below the table is indicated the mean value of effective migration rate of each sample, respect to the remaining

	Ig	Pe	Nn	Ca	Yj	Cj	Lo	Me	US	Ec
Ig		0.1487	0.1932	0.1617	0.2142	0.2189	0.2564	0.1203	0.3379	0.4350
Pe	0.206± 0.239 (1)		0.0922	0.1401	0.1396	0.0818	0.0980	0.2226	0.5323	0.4432
Nn	0.237± 0.39 (0.8)	0.164± 0.209 (1.3)		0.1679	0.1812	0.0750	0.0847	0.2128	0.5074	0.4545
Ca	0.201± 0.280 (1)	0.203± 0.267 (1)	0.230± 0.251 (0.8)		0.0487	0.2407	0.1883	0.2311	0.4085	0.5962
Yj	0.279± 0.291 (0.6)	0.246± 0.315 (0.8)	0.288± 0.288 (0.6)	0.460± 0.432 (0.3)		0.2486	0.2083	0.2798	0.5379	0.6076
Cj	0.533± 0.287 (0.2)	0.178± 0.193 (1.2)	0.546± 0.420 (0.2)	0.335± 0.308 (0.5)	0.522± 0.344 (0.2)		0.0480	0.2514	0.5787	0.4680
Lo	0.311± 0.297 (0.6)	0.599± 0.397 (0.2)	0.181± 0.178 (1.1)	0.280± 0.300 (0.6)	0.658± 0.370 (0.1)	0.522± 0.344 (0.2)		0.2411	0.5611	0.4995
Me	0.146± 0.218 (1.5)	0.424± 0.361 (0.3)	0.253± 0.262 (0.7)	0.245± 0.277 (0.8)	0.475± 0.348 (0.3)	0.506± 0.275 (0.2)	0.578± 0.274 (0.2)		0.3054	0.4192
US	0.282± 0.339 (0.6)	0.481± 0.366 (0.3)	0.543± 0.308 (0.2)	0.341± 0.351 (0.5)	0.429± 0.357 (0.3)	0.438± 0.364 (0.3)	0.429± 0.364 (0.3)	0.239± 0.315 (0.8)		0.5294
Mean Nm	0.78	0.76	0.71	0.68	0.40	0.37	0.41	0.60	0.41	

the most genetically differentiated, which is consistent with its isolation as a separate species. Within *A. grandis*, the group of samples from South America is genetically more similar to the sample from Mexico, than to that one from the United States. However, since Mississippi is a laboratory reared sample, it could be not representative of all the genetic variation present in natural populations (Terranova, Jones & Bartlett, 1991). Bootstrap values shown at the nodes of the neighbor-joining tree, indicate that most relationships among populations are well supported (values over 50%).

All populations from South America are genetically distinct from each other. Those from the same country, show the highest degree of genetic similarity, except the sample from Iguazú National Park (Argentina). This sample, collected on native plants, is more similar to the geographically distant population from Tecomán (Mexico), near the original area of distribution of the boll weevil, than to the remaining South American populations (Figure 3). The high values of polymorphic loci reach by both populations, are characteristic for ecologically central populations (Brussard, 1984) (Table 3).

In order to provide further insights into this problem, fixation indices (Fst) and effective migration rates (Nm) were estimated (Table 4, below the diag-

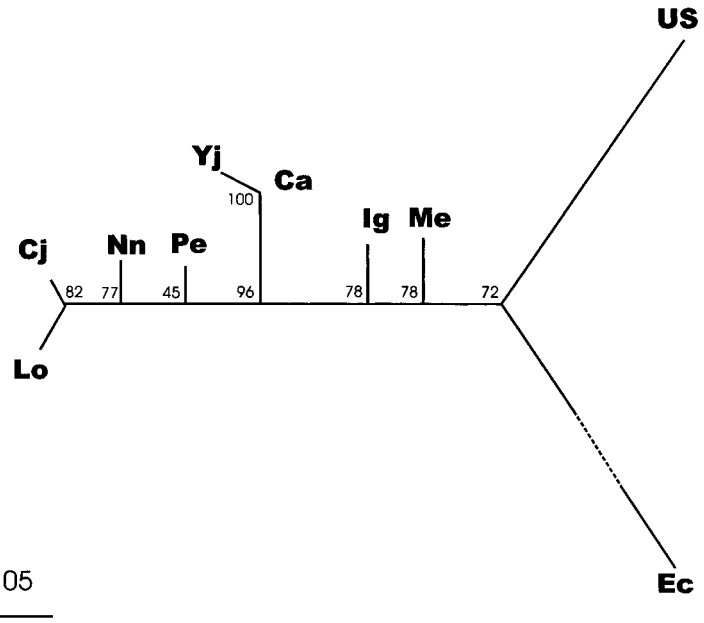

Figure 3. Neighbor-joining tree of *A. grandis* samples and *A. vestitus*. Acronyms according to Table 1. Numbers at the nodes correspond to bootstrap values. Scale of 0.05, indicates genetic distances.

onal). Indices were statistically significant indicating that all samples behave as genetically differentiated independent populations. The estimates of gene flow among all pairs of populations, are not always correlated with their geographic distances. Indeed, the pair Tecomán (Mexico)-Puerto Iguazú (Argentina), showed the highest value of effective migration rate (1.5 migrants per generation).

In a further analysis, we calculate the mean effective migration rate of each population, to infer the relative relationships of all samples (mean value among all estimations of gene flow, for each sample respect to the others). Populations with high mean values (Nm), are expected to have a central position, whereas populations with low mean values, will reflect their marginal position regarding central populations. Interestingly, samples from Misiones province (Puerto Iguazú and Puerto Península), collected on native plants, show the highest mean values of migration rates (Table 4, bottom).

Correlation between genetic and geographic distances among all populations gave a significant result ($r = 0.67$; $p = 0.0057$) as expected for neutral loci. However, when we considered only South American populations, this correlation was not statistically significant ($r = 0.18$; $p = 0.22$) probably due to the great genetic differentiation of Puerto Iguazú sample, with respect to remaining populations.

Discussion

Except for some special cases, RAPD markers are considered neutral loci, so, a positive correlation between genetic and geographic distances is expected (Confalonieri, 1999; Confalonieri, Scataglini & Remis, 2000). When there is not such a correlation, as in the pair Tecomán-Puerto Iguazú, a further explanation should be given (Confalonieri, 1994; Matrajt, Confalonieri & Vilardi, 1996).

The genetic similarity of these two geographically distant populations, could be the result of either a secondary adaptation to a similar host plant, or to an ancestral relationship between both populations. Since the sample from Mexico has been collected on cotton, and the population from Misiones occurs on native Malvaceae, genetic similarity of Tecomán-Puerto Iguazú populations would be not a result of an adaptation to similar host plants. Moreover, an hypothesis of secondary adaptation of boll weevils coming from cotton cultures, to native Malvaceae of the Iguazú National Park, does not explain the high percentage of polymorphic loci of this population. Consequently, we propose the hypothesis of an ancestral relationship of populations of Tecomán and Puerto Iguazú, and that boll weevil has occurred in wild areas of South America, prior to the extensive cotton cultivation in the continent.

The high percentage of polymorphic loci is typical of original central populations, and on the contrary, low values of polymorphic loci characterize recent insect pest populations. The fact that Tecomán is close to the tropical environment proposed as the area of origin of the boll weevil (Monclova, Coahuila), where there is the highest diversity of noncultivated host plants (Burke et al., 1986), is coincident with the high polymorphism of its populations. Puerto Iguazú National Park is a subtropical area of South America, where several Malvaceae of the genera *Cienfuegosia, Thespesia* and *Hibiscus* would behave as hosts of the boll weevil (Manessi, 1997). It sounds logical that natural populations occurring in that area, have higher percentages of polymorphism in comparison with remaining South American populations, ranging in cultivated cotton lands.

The capacity to adapt to different host plants, has been a key factor for the dispersal of *A. grandis*, in prehistoric times (Burke et al., 1986). As a matter of fact, it is the only species of the *grandis* group that was able to extend its geographic range far beyond its area of origin and to reproduce in other Malvaceae than *Hampea (Gossypium, Cienfuegosia, Thespesia* and *Hibiscus*) (Burke, 1968; Burke & Cate, 1979; Burke et al., 1986; Jones & Burke, 1997). Consequently, it sounds likely that the boll weevil was able to disperse naturally, to tropical and subtropical areas of South America, where climatic conditions are suitable for its development and wild host are available.

After the Isthmus of Panamá arose at the end of Pliocene times, there was an extensive exchange of faunas between North and South America. In the absence of a geographical barrier for terrestrial animals, several South American species reached the subtropical rainforests of Mexico and Central America, and many species from that area have dispersed into tropical South America (Halffter, 1964, 1974; Confalonieri et al., 1998). The present geographical range of the boll weevil, *A. grandis,* is similar to the natural range of many insect species best adapted to lowland tropical conditions.

There are early records of the occurrence of the boll weevil in South America, associated to natural vegetation, however, it was interpreted that specimens collected on wild Malvaceae migrated from cotton cultures. *Anthonomus grandis* was first reported from Venezuela in 1949, and it was collected on the native host *Cienfuegosia affinis* in 1952, occurring also in Venezuela and Colombia on *Thespesia populnea* (Cross et al., 1975). In recent years, Lanteri (Pers. Comm.) identified specimens of *A. grandis* from Salta province (Argentina) and Santa Cruz de la Sierra (Bolivia) collected far from cotton lands. This area corresponds to the subtropical forest of the Yungas (Cabrera & Willink, 1980), harboring several plant species of Gossypieae, potential hosts of the boll weevil.

We suspect that the presence of *A. grandis* in South America was ignored until 1949, due to the scarce weevil collectings, especially on native areas, the lack of systematic knowledge of South American Anthonomine (Clark & Burke, 1986, 1996), and to the absence of populations outbreaks associated to cotton cultivation. Manessi (1997) noticed that when *A. grandis* eats and reproduces on *Gossypium hirsutum* it produces more agregations pheromone, and Fryxell & Lukefahr (1967) remarked the importance of the expansion of cotton production in allowing the boll weevil to increase their populations and to extend its range in the US Cotton Belt. It is probable that the dispersal of the boll weevil in vast areas of South America, during historic times, was associated to the increase of agricultural cotton lands, same as in North America.

There are other phytophagous insects distributed in arboreal savannas of Brazil that became pests at the beginning of the 1980 decade, probably due to the outstanding increase of agricultural activities. For example, the grasshopper *Rhammatocerus schistocercoides* (Acrididae), that was only known for scientists and had been recorded for Mato Grosso states and vicinities, until 1983. After that, and due to its agricultural importance, entomological studies and collecting increased. Consequently, it was found that the species ranges from Mexico throughout Central America, Colombia, Perú and Bolivia, down to southern Brazil and Uruguay (Miranda et al., 1996).

Finally, we want to address the question on source populations of the boll weevils that affect cotton in Brazil, Paraguay and other South American countries. According to Burke et al. (1986) Brazilian specimens are morphologically similar to the Southeastern form of *A. grandis*, distributed in northeastern Mexico and southeastern US. We noticed that whereas specimens from Brazil and Paraguay are similar to the Southeastern form, specimens from Puerto Iguazú, are much larger, and closer to the Mexican form. Consequently, it is possible that South American boll weevils have different origins. Some populations occurring in natural areas, would be ancestral and associated to the Mexican form, as it is suggested by the genetic sim-

ilarity and high variability of Tecomán-Puerto Iguazú samples. Other populations, associated to cotton cultures, would be related to the Southeastern form, as it was proposed by Burke et al. (1986). Results from RAPD analysis does not provide support to the hypothesis of relationship between the Southeastern boll weevil and the populations affecting cotton in South America. However, as the Mississippi sample studied by us is a reared colony, we were not able to test this hypothesis properly.

Prospects

Results of RAPD technique provide useful information on the current levels of gene flow between different populations of the boll weevil, and shed light on the understanding of the origin and dispersal of this important agricultural pest in South America. These results will assist further research on this subject and may be suitable for more detailed molecular analyses.

The hypothesis herein proposed, that *A. grandis* occurs in South America since prehistoric times, is being tested by mitochondrial DNA studies of the boll weevil populations from different South American countries and natural populations from Mexico and the United States, considered as putative sources of origin. We also expect to be able to compare samples from populations infesting cotton and developing on native plants, in order to test hypotheses about time and frequency of host shifts between them.

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