Genetica (2007) 131:11–20
DOI 10.1007/s10709-006-9108-3

ORIGINAL PAPER

Population structure of the boll weevil in cotton fields and subtropical forests of South America: a bayesian approach

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Received: 21 April 2006 / Accepted: 31 August 2006 / Published online: 17 October 2006

Abstract The main goal of this contribution is to investigate the genetic structure of boll weevil populations from South America (Argentina and Brazil) and to make further comparisons with a putative source population from USA. Samples were collected in a Paranaense forest under reserve protection, cotton fields and non-cultivated areas. Data from anonymous molecular markers were analysed using both traditional methods of population genetics and Bayesian approaches. Results help to support a previous hypothesis on the presence of two lineages of boll weevil populations in South America: one with characteristics of recent invaders and the other with characteristics of ancient populations. The sample from Urugua-í Provincial Park (Misiones, Argentina) shows the highest percentage of polymorphic loci, the highest values of mean heterozigosity, and the largest number of population-specific alleles, all being typical features of ancient populations. Furthermore, the Urugua-í sample shows two gene pools occurring in sympatry, probably as a consequence of a secondary contact. The remaining samples reveal not only lower percentages of polymorphic loci and heterozygosity values, but also an almost negligible presence of specific alleles. Bayesian methods also suggest the occasional migration of some individuals of ancient lineages from their natural habitats in fragments of the Paranaense forest into cotton fields, and vice versa.

Keywords Boll weevil · RAPDs · Genetic structure · Bayesian approach · *Anthonomus grandis*

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Introduction

The ''boll weevil'' *Anthonomus grandis* Boheman (Coleoptera, Curculionidae) is the most harmful insect pest of cotton in the Americas, causing severe fruit losses and boll damages (Lanteri 1999). Its original area of distribution, ancestral host plants and pathways of dispersal throughout the continent have been subject of debate during the last century (Townsend 1895; Fryxell and Lukefahr 1967; Burke and Cate 1979; Burke et al. 1986; Jones and Burke 1997; Scataglini et al. 2000,2006).

Some authors proposed the hypothesis that the boll weevil originated in southern Mexico, from where it spread to USA together with its wild host plants (Malvaceae of the tribe Gossypieae) probably during the Pleistocene (Burke et al. 1986; Jones 2001). Once in USA, *A. grandis* spread out rapidly throughout the Cotton Belt from 1889 to 1916 by the extension of cotton culture.

There is little information on the appearance and dispersal range of *A. grandis* in South America. On the basis that some boll weevil specimens in South America are morphologically similar to those in

southern USA, Burke et al. (1986) proposed the occurrence of multiple introductions from this country associated with commercial exchange. However, genetic studies using Random Amplified Polymorphic DNA markers (RAPD) and a phylogeographic approach based on mitochondrial DNA sequences have given support to a new hypothesis on the origin of South American boll weevils, that could change the traditional viewpoints about the evolutionary history of the species (Scataglini et al. 2000,2006; Confalonieri et al. 2003; Lanteri et al. 2003). This novel hypothesis suggests that boll weevil populations in South America can be classified into two types: (i) populations with a single haplotype or very few haplotypes, which are remnants of ''bottlenecks'' that occurred after colonization, and with characteristics of ''recent invaders'' introduced by trade; and (ii) populations with several highly differentiated haplotypes, which represent an ''ancient population'' present in the continent before cotton cultivation. The former type is associated to cotton fields and/or nearby areas, and the latter to pristine areas with native forests, such as the Iguazú National Park, Misiones Province, Argentina (Scataglini et al. 2000, 2006).

The Paranaense forests, distributed in southern Brazil, west of ''Serra do Mar'', northeastern Argentina and eastern Paraguay (Cabrera and Willink 1980), are considered high-priority areas for conservation due to their richness in biodiversity and genetic resources (Bibby et al. 1992). However, intensified agricultural practices and increasing urbanization during the last 25 years reduced these forests to about 45% of their original extent, turning them into discrete, partially isolated fragments (Laclau 1994).

The first record of the boll weevil in Argentina was reported from Misiones, Iguazú National Park in 1993 and since then, the species has been found over the entire province despite of the absence of cotton cultures. This situation may have been due to the migration of boll weevils from cotton fields of neighbouring areas to Misiones, and/or from fragments of pristine forests of this province to surrounding disturbed areas. Scataglini et al. (2006) proposed that in Misiones there are boll weevil populations with characteristics of recent invaders and ancient populations. If this is the case, it is expected that boll weevil lineages from pristine forests of the same province will be similar to those in Iguazú National Park.

Do ancient boll weevils and recent invaders occur in allopatry or are they in sympatry as a consequence of a secondary contact? Are individuals of ancient populations invading cotton cultures? Are recent invaders also found in pristine forests or neighbouring areas? The answers to these and other questions are relevant not only to provide a complete scenario of the evolution of the boll weevil, but also to establish more effective strategies for pest management in the area under study.

In order to answer the previous questions, we decided to investigate the genetic structure of some boll weevil populations from native forests and cotton fields of Argentina and Brazil, and a potential source population of USA, using anonymous molecular markers and Bayesian approaches.

Despite the usefulness of molecular markers to examine gene flow levels among populations and to propose hypotheses on ancestral areas and routes of migrations of insect pests, they have just recently been applied in boll weevil studies (Roehrdanz 2001; Roehrdanz and North 1992; Scataglini et al. 2000, 2006; Kim and Sappington 2004).

Until recently, the dominant nature of molecular markers was regarded as a potential drawback for population studies (Baus et al. 2005) because methods for partitioning genetic diversity require the assumption of Hardy-Weinberg equilibrium and the knowledge of population inbreeding coefficients (Lynch and Milligan 1994; Zhivotovsky 1999). However, the currently available Bayesian approaches (Holsinger et al. 2002) allow the incorporation of uncertainty in the magnitude of within-population inbreeding coefficients, in order to estimate F_{ST} and other diversity parameters. In addition, Bayesian techniques are being applied to multilocus data to infer population genetic structure and to define the number of clusters (=gene pools) of a given data set (Pritchard et al. 2000). In the case of the boll weevil, we expect that the application of these modern statistical tools will contribute to identify mixed populations (composed of individuals belonging to different inferred gene pools) and/or to detect foreign genotypes within each population. In turn, we may help to shed light on the evolutionary history of this important insect pest.

Materials and methods

Sampling

Seven population samples of *A. grandis* from Argentina, Brazil, and USA were analysed. Localities of collection and sample sizes are shown in Fig. 1 and Table 1. Specimens from Laguna Naick Neck (Formosa, Argentina), Jatalzhino and Juranda (Paraná State, Brazil) and Lubbock (Texas, USA) were collected near cotton fields. The remaining three

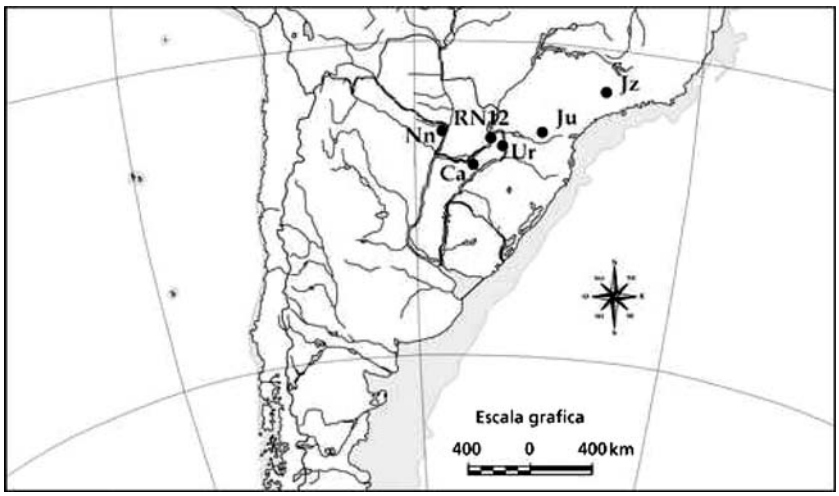

Fig. 1 Map showing the geographic locations of *Anthonomus grandis* samples from South America. Acronyms according to Table 1

samples came from Misiones (Argentina) and were taken from non-cultivated areas at locations with different characteristics as follows: Urugua-í is a provincial park with pristine Paranaense forest, located in northeastern Misiones and southwest to Iguazú National Park, with which it is connected by a ''green corridor''; Ruta Nacional 12 is a highway on the west side of the Iguazú National Park that represents a highly disturbed environment; and Candelaria, located in southwestern Misiones, represents a non-protected forest.

Specimens were collected in traps containing 'glandure' pheromone (Manessi 1997). All insects studied were preserved in 100% ethanol, but only live weevils dropped into ethanol just after being trapped were suitable for DNA analyses.

DNA isolation and amplification

DNA was isolated from insects according to the method applied by Reiss et al. (1995). The conventional RAPD technique was modified using two different decamer primers simultaneously, in order to analyze a larger number of fragments (Welsh and McClelland, 1990; Hu and Quiros, 1995; Sall et al. 2000). Pairs of primers were selected using Qiagen Oligo Analysis and Plotting Tool (http://oligos.qiagen.com/oligos/toolkit.php). Five combinations were analysed, but only one produced discernable and repeatable bands (OPB–09 5́TGGGGGACTC/OPB-10 5́CTGCTGGGAC, from Operon Technologies).

Amplification conditions were based on Welsh and McClelland (1991) and Sall et al. (2000). The reaction was performed in 10 X Buffer (Invitrogen), 4 mM MgCl2 (Invitrogen), 100 mM of each dNTP, 0.8 μM of each primer, 50–100 ng of total genomic DNA and 1 unit of *Taq* polymerase (Invitrogen) in final volume of 25 μl. DNA amplification was done in a thermal cycler (Eppendorf Mastercycle). The first period of denaturation was 96°C for 6 min, followed by 40 cycles of denaturation (96°C for 1 min), annealing (36°C for 1 min) and extension (72°C for 2 min), with a final extension cycle of 72°C for 4 min. For each series of amplifications one negative control (absence of template) was performed.

A high-resolution procedure was applied to resolve the high number of RAPD bands obtained with the two different decamer primers. The amplification products were separated through 6% (w/v) denaturing polyacrylamide gels. Electrophoresis was carried out in 1X TBE buffer at constant power (60 W) and room temperature for 1–2 h. The fragments were detected by silver staining (Silver Sequence™ DNA Staining, Reagents, Promega, USA), following the instructions of the manufacturer.

Table 1 Description of the samples collected from seven populations of *Anthonomus grandis*. *N* = sample size

Localities	Acronyms	States/Countries	Vegetation	*N*
Candelaria	Ca	Misiones/ Argentina	Non cultivated area	8
Ruta Nacional 12	RN12	Misiones/ Argentina	Non cultivated area	10
Urugua-í	Ur	Misiones/ Argentina	Pristine forest area	11
Laguna Naick Neck	Nn	Formosa/ Argentina	Cotton Fields	14
Jatalzhino	Jz	Paraná/Brazil	Cotton Fields	10
Juranda	Ju	Paraná/Brazil	Cotton Fields	10
Lubbock	Tx	Texas/USA	Cotton Fields	10

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. Amplified fragments per individual were recorded as present (1) or absent (0). Since we analysed intraspecific variation, reproducible products that comigrated were assumed to represent homologous loci, each one with two alleles with dominance relationship. Therefore, we supposed that the percentage of codominant loci was too low to affect estimations of allele frequencies, and that possible errors were compensated by the large number of loci analysed.

Parameters of population diversity and structure were estimated using different approaches. Allelic frequencies and heterozygosities were first calculated with the computer programs RAPDBIOS 2.0 (Black 1997) and BIOSYS-1 1.7 (Swofford and Selander 1981), assuming that genotypes are in Hardy-Weinberg equilibrium. Lynch and Milligan (1994) correction for small sample sizes was applied when estimating allelic frequencies. Levels of differentiation among populations were estimated through the Fst index (Weir and Cockerham 1984) using the RAPDFST program (Black 1997), which includes a correction for small and unequal sample sizes.

The matrix of amplified fragments per individual was used to create a dissimilarity matrix among individuals (Nei 1972), using RAPDPLOT 3.0 (Black 1997), that was analysed by the Neighbour-Joining method (Saitou and Nei 1987), with the NEIGHBOUR and CONSENSE programs of the PHYLIP 3.5c package (Felsenstein 1993). Branch supports of the obtained tree were estimated using a bootstrap analysis with 700 replications.

A Bayesian statistical procedure was applied to investigate levels of heterozygosis and population structure with dominant markers (Holsinger et al. 2002). This hierarchical approach allows analysis of genetic data incorporating the effect of uncertainty on the estimation of the fixation index (θ_B, a Bayesian analogue of F_{ST}), on the inbreeding coefficient (f, a Bayesian analogue of F_{IS}), and on the heterozygosity indices, using a Markov Chain Monte Carlo procedure (MCMC). The latter was run 250,000 times with the HICKORY v 1.0 computer program (Holsinger et al. 2002) to ensure convergence of Markov chains to their stationary distribution and, after discarding the first 50,000 simulations, estimations every five steps were retained to avoid autocorrelation among samples. The Deviance Information Criterion (DIC) (Spiegelhalter et al. 2002) was used to assess how well a particular model fits the data, and to choose among different models: (i) θ_B and $f \neq 0$ (the full model); (ii) $\theta_B = 0$ and $f \neq 0$; (iii) $\theta_B \neq 0$ and $f = 0$.

A Bayesian clustering method implemented in the STRUCTURE v2.1 computer program (Pritchard et al. 2000) was used for further examination of population structure and to assign probabilistically individual genotypes to a user-defined K number of clusters. It accounts for the occurrence of Hardy–Weinberg or linkage disequilibrium by introducing population structure and attempts to find population groupings that, as far as possible, are not in disequilibrium (Pritchard et al. 2000). Thus, the method estimates the most appropriate number of populations (K) needed to interpret the observed genotypes and calculates the probability of individual assignments to population clusters. As it is not possible to distinguish heterozygote individuals from RAPD fragments, we considered each genotype as being a haploid allele as suggested by Pritchard et al. (2000). STRUCTURE was run twenty times for each K, from $K = 2$ to $K = 15$ for 500,000 iterations, after a burn-in period of 100,000 iterations without any prior information on the population origin of each individual. We applied the model of no admixture under the assumption that each individual originates purely from one of the populations. Furthermore, allele frequencies were kept independent among clusters ($\lambda = 1$). The probability of the number of populations equalling K was calculated from the estimated log probability of the data and the number of populations with the highest posterior probability was identified.

Results

Genetic diversity within populations

Seventy-three individuals of *Anthonomus grandis* sampled from seven populations were scored for high-resolution RAPD markers. The selected pair of primers revealed 77 bands that could be unambiguously scored in blind tests. These products, which ranged in size between 167 and 1250 bp, were retained for further analyses (Fig. 2).

The boll weevil populations showed a considerable amount of variation (Table 2). The percentage of polymorphic bands (P) ranged from 18.2% for Juranda to 77.9% for Urugua-í Provincial Park. The estimated mean heterozygosity ranged from 0.060 for the Juranda sample to 0.346 for the Urugua-í sample. The latter, along with the Candelaria sample, were the only ones showing population-specific alleles in South America (A). The mean heterozygosity value from samples collected in non-cultivated areas was higher ($H = 0.20$)

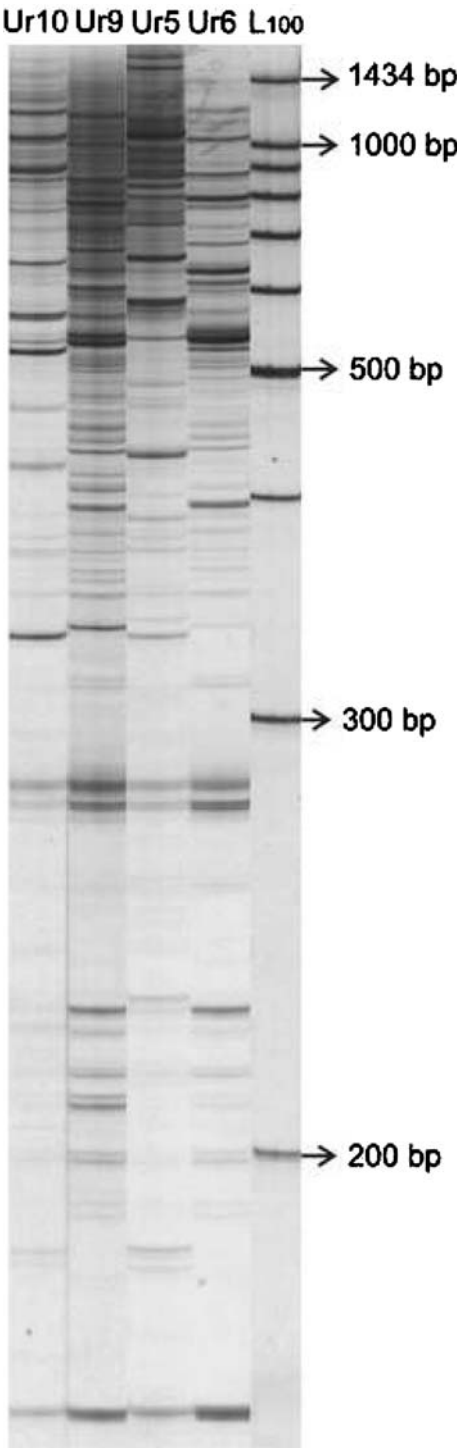

Fig. 2 An example of the RAPD fragments amplified in Urugua-í population of *A. grandis*. Acronyms according to Table 1; L100: Ladder containing fragments from 100 to 1000 bp with an aditional band of 1434 bp

than that in cotton-cultivated areas ($H = 0.125$), but some of the latter (e.g. Laguna Naick-Neck) showed higher levels of variability than others from non-cultivated areas.

The Bayesian estimates of mean heterozygosity (H_B) showed a distribution pattern among populations similar to that observed using conventional estimates (H). Notwithstanding, H_B values were higher than H values, particularly for the Laguna Naick Neck sample, and ranged from 0.1672 for the Ruta Nacional 12 sample to 0.3481 for the Urugua-í sample. These H_B scores were obtained with the ''full model'', because it recorded the lowest DIC value (DIC = 1185 for the full model, DIC = 1191 for the $f = 0$ model, DIC = 2375 for $\theta_B = 0$ model).

Genetic similarity of samples

In the obtained Neighbour-Joining tree (Fig. 3), most individuals were grouped according to their collection site. Specimens from Texas (USA) formed a group (percent bootstrap value = 86.8%), as well as specimens from Ruta Nacional 12 (Misiones) (77%), Candelaria (66.6%) and Juranda (55%). Four individuals from Urugua-í Provincial Park (Ur4, Ur5, Ur10 and Ur11) formed a significant cluster (74.4%), to which an individual from Laguna Naick (Nn3) with a significant bootstrap support (72.1%) was joined.

Population structure

In the Bayesian analysis of population structure, the criterion to choose the full model over the $f = 0$ model, would indicate that genotype frequencies within populations deviate from Hardy-Weinberg expectations. In fact, the posterior mean estimate for f was slightly higher than 0 ($f = 0.0986 \pm 0.666$; 95% credible intervals 0.0059 and 0.2573). However, this assumption should be considered with caution because the DIC value obtained with the full model was only six units smaller than that with the $f = 0$ model.

The posterior mean estimates of θ_B, under the full model and under the $f = 0$ model were similar ($\theta_B = 0.3284 \pm 0.0254$; 95% credible intervals 0.2780 and 0.3822), but slightly higher than the traditional estimate of the overall F_{ST} value among all populations ($F_{ST} = 0.3023 \pm 0.0342$; $p = 0.001$). However, both Bayesian (DIC $\theta_B = 0$>> DIC full model and DIC $f = 0$ model) and F_{ST} estimates of population structuring strongly suggested that populations were highly differentiated and behaved as independent units.

Using the model-based clustering method of Pritchard, Stephans and Donnelly (2000), the highest estimate of the likelihood of the data, conditional on a given number of clusters (K), was obtained when clustering all genotypes into eight gene pools (one more than the number of populations analysed).

Table 2 Parameters of genetic diversity estimated on seven populations of *Anthonomus grandis*. P(%) = percentage of polymorphic loci. H = mean heterozygosity estimated assuming Hardy-Weinberg equilibrium. H_B = mean heterozygosity estimated through Bayesian statistical techniques. A = population-specific alleles

Localities	P (%)	H	H_B	A
Candelaria	37.3	0.169±0.020	0.2333±0.0162	2
Ruta Nacional 12	22.1	0.084±0.020	0.1672±0.0161	–
Urugua-í	77.9	0.346±0.026	0.3481±0.0131	8
Laguna Naick Neck	42.9	0.178±0.024	0.3301±0.0143	–
Jatalzhino	26.0	0.118±0.023	0.1929±0.0164	–
Juranda	18.2	0.060±0.017	0.1831±0.0186	–
Lubbock	32.5	0.144±0.025	0.2105±0.0160	1

Figure 4 shows how individuals belonging to the seven samples under study were split into successive clusters, (indicated by different screen lines). When $K = 8$ was reached, each population approximately corresponded to one of the inferred gene pools, except for Urugua-í and Laguna Naick- Neck. In fact, from $K = 4$ onwards, Urugua-í became substructured into two clusters, hereafter referred to as UR1 and UR2. The latter group included the same individuals clustered with significant bootstrap support in the NJ tree (Fig. 3): Ur4, Ur5, Ur10 and Ur 11, from Urugua-ì, and Nn3 from Laguna Naick-Neck.

The analysis of the successive splitting of individuals into different K values may also suggest that individuals from Candelaria and UR1 remain together until $K = 7$, indicating an ancient genetic affinity between these two gene pools. On the other hand, at $K = 4$ UR2 was

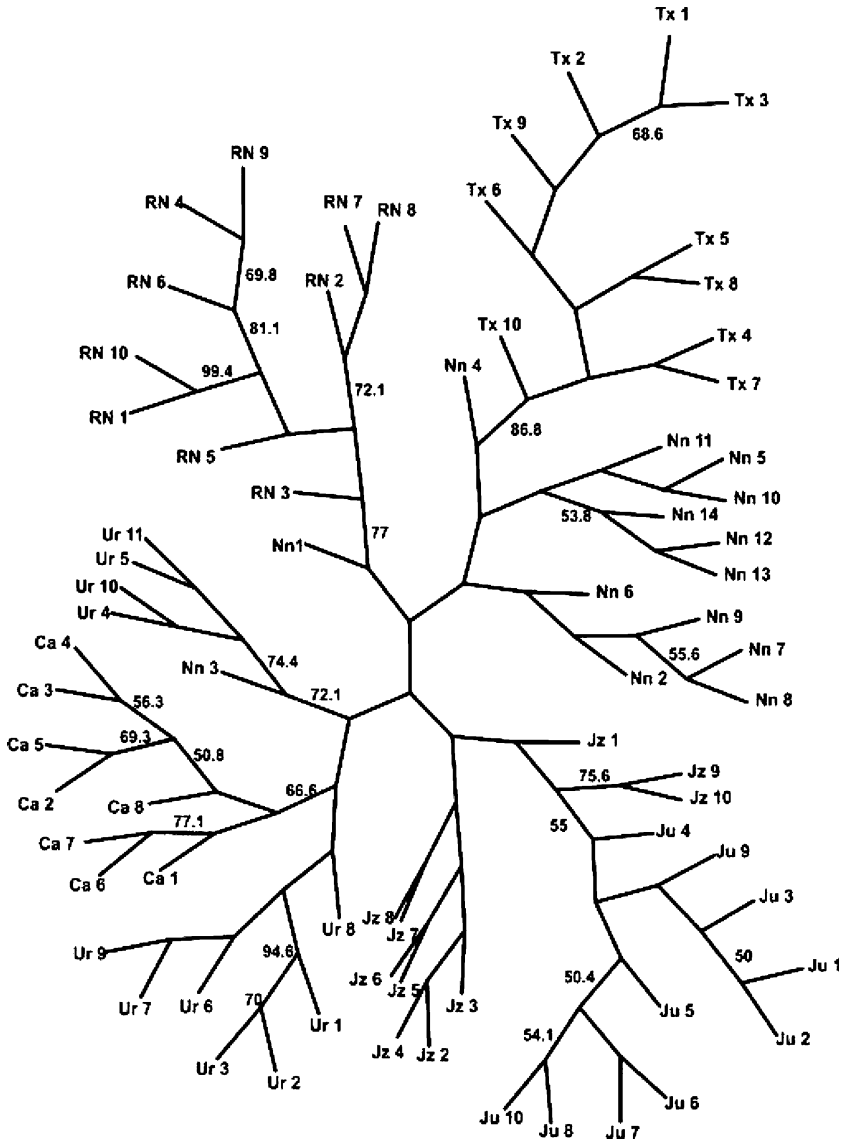

Fig. 3 Neighbour -Joining tree showing the genetic relationships among all individuals studied. Acronyms according to Table 1 including numbers to identify individuals. Numbers on the branches indicate boostrap values

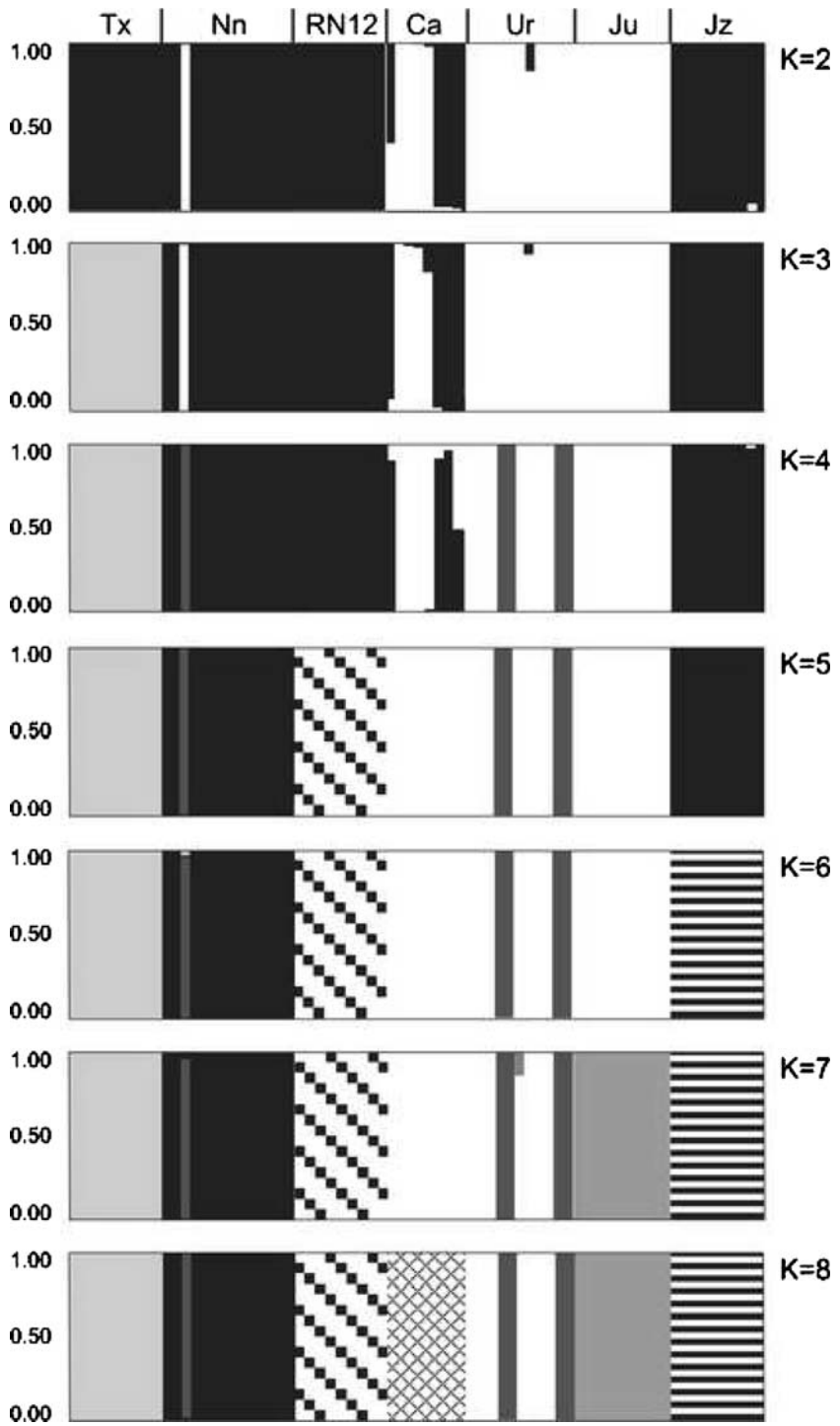

Fig. 4 Estimated population structure. Each individual is represented by a thin vertical segment. Blocks represent groups of individuals of different gene pools, according to the individual's estimated membership fractions of K, and they are indicated by different line screens. Populations are separated by vertical lines above the figure, and are identified by acronyms according to table 1. Eight structure runs at each K produced nearly identical individual membership coefficient

separated not only from UR1 but also from the remaining samples, suggesting that in Urugua-í there were sympatric individuals from two different gene pools as can also be inferred from the NJ tree.

On the basis that the model-based clustering method attempts to find populations that are not in Hardy-Weinberg disequilibrium (Pritchard et al. 2000), and that most of the populations corresponded to inferred gene pools, it can be concluded that they did not deviate from the Hardy-Weinberg equilibrium, except for the Urugua-í and Laguna Naick- Neck samples. This result is congruent with the other Bayesian analysis of population structure implemented here, because the difference of only six DIC units favouring the full model over the $f = 0$ model, is most probably accounted for by the two clusters of Urugua-í (UR1

and UR2) and the single individual from Laguna Naick-Neck that clustered into UR2.

The fact that Urugua-í and Laguna Naick-Neck were composed of two different gene pools explains the high values of heterozygosity and the high proportion of polymorphic loci found in these samples. If mean heterozygosis were re-calculated considering eight populations and including the single individual collected in Laguna Naick-Neck (Nn3) into the cluster UR2, the Nn sample would show lower values of H (Nn: $H = 0.105 \pm 0.021$), as well as the samples UR1 and UR2 (UR1: $H = 0.203+/-0.026$; and UR2: $H = 0.317 \pm 0.026$). According to this re-calculation, the highest values of mean heterozigosity will correspond to UR1, UR2 and Candelaria.

On the other hand, Urugua-í showed eight population specific alleles, of which four were shared by UR1 and UR2, thus indicating their ancestral affinity, one was exclusive of UR1 and three were exclusive of UR2. If the single foreign individual from Laguna Naick-Neck (Nn3) is included in UR2, the number of private alleles of the latter will be seven because the referred individual contributes with four private alleles.

Discussion

About the presence of ancient boll weevils lineages in South America

The results of the Bayesian analyses confirm previous results obtained with other molecular markers on the existence of boll weevil populations with characteristics of ancient lineages, which are associated with pristine forests (Scataglini et al. 2000, 2006). In the paper by Scataglini et al. (2006), the sample from Iguazú National Park (Misiones, Argentina) showed the highest haplotypic diversity and did not share any haplotype with populations outside the park, despite their geographical proximity. In the present study, the only sample associated with a pristine environment of the Paranaense forest was that from Urugua-í Provincial Park, geographically close to Iguazú and connected with it through a green corridor. The Urugua-í sample shows the highest percentage of polymorphic loci, the highest values of mean heterozygosity, and the maximum number of population-specific alleles, the latter being typical of ancient populations. The variability of the Candelaria sample, collected in an area of native vegetation in southwestern Misiones with a moderate anthropogenic impact, shows intermediate levels of polymorphic loci and heterozygosity, and is characterised by two private alleles.

According to Bayesian analysis, Candelaria is grouped with Urugua-í until $K = 4$, being both samples associated with distantly separated fragments of the Paranaense forest. The remaining samples, associated with cotton fields or non-cultivated areas with different degrees of anthropogenic disturbance, revealed not only lower percentages of polymorphic loci and heterozygosity values, but also an almost negligible presence of specific alleles.

About the genetic structure of ancient boll weevils samples

The sympatry of the two divergent lineages (or subpopulations) in the Urugua-í Provincial Park is presumably due to a secondary contact, and the absence of geographical barriers led us to suspect that they evolved allopatrically in different forest fragments. The cyclical fragmentation and reconnection events taking place in the subtropical forests have had a great influence on the evolution of the South American biota. In geological times, it was affected by the wet-dry cycles during the Plio-Pleistocene (Avise and Walker, 1998) and in historical times, by the deforestation and reconnection of forest reserves through green corridors. The genetic structure of the Urugua-í sample, composed of two lineages that may have separated a long time ago, can be considered a good example of past fragmentation events resulting from changes in climate conditions.

Are individuals of ancient lineages invading cotton fields?

The presence of one individual from Urugua-í (Misiones) in Laguna Naick- Neck (Formosa) helps to support the hypothesis on boll weevil migration from pristine forests to cotton-fields. In previous analyses using mitochondrial DNA, the samples from cotton-fields of Formosa, as well as those from the neighbouring country Paraguay, were monomorphic for the haplotype ''A'', which is the most frequent haplotype among cotton samples of North and South America (Scataglini et al. 2006). For this reason it is somewhat surprising that in the present analyses the levels of mean heterozygosity and polymorphic loci of the Formosa sample are quite high when compared to those of the remaining samples (all except Urugua-í). This could be explained by the migration of individuals from areas of native vegetation to cotton fields, as suggested by the results from the population structure analysis. In this regard, it is worth noticing that Formosa Province does not

belong to the Paranaense Biogeographic Unit but to the Chaco Biogeographic Unit, which is characterised by xerophytic forests with several native host plants for the boll weevil, especially those of the genus *Cienfuegosia* (Krapovickas 2000.

Are recent invaders found in areas with pristine forests?

The sample collected near a highway of Misiones Province (RN12, Ruta Nacional 12), close to the Iguazú National Park, had low values of heterozygosity and the lowest percentage of polymorphic loci. It is a highly disturbed area of the Paranaense forest bearing boll weevils with genetic characteristics of recent invaders. Similar results were obtained by Scataglini et al. (2006) for populations from Puerto Península (northwestern Misiones, outside of the Iguazú National Park), which were genetically similar to the samples collected in cotton fields. Consequently, recent invaders experiencing a secondary contact with ancient lineages, are indeed found in disturbed areas neighbouring the Paranaense forest.

Concluding remarks

In regard of the evolutionary scenario of the boll weevil, our present data further support our previous hypothesis on the presence of ancient lineages of *A. grandis* in South America. This is the most plausible explanation for the occurrence of populations with a large number of specific alleles associated with subtropical forests of Misiones, whose genetic variability parallels that of populations from the proposed original area of distribution of the species, namely the humid forest of southern Mexico (Scataglini et al. 2000, 2006).

The Urugua-í sample was collected in the Paranaense forest, a high-priority area for conservation due to its richness in biodiversity and genetic resources (Bibby et al. 1992). Our data clearly demonstrate that individuals coming from this protected area, with genetic characteristics of ancient lineages, can also be found in neighbouring cotton fields.

The reduction of the Paranaense forest caused by intensive agriculture most probably favoured the emigration of boll weevils from pristine areas, a fact that should be taken into account for the development of effective pest management strategies in the region.

Acknowledgements This work was supported by grants from the Argentina's National Research Council (CONICET), the University of Buenos Aires.

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