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Genetic Variability of Five *Periplaneta americana* L. (Dyctioptera: Blattidae) Populations in Southwestern Colombia Using the AFLP Molecular Marker Technique

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ABSTRACT - The genetic structure of five *Periplaneta americana* (L.) populations from three cities (Cali, Popayán and Buenaventura) located in southwestern Colombia was estimated using the AFLP molecular marker technique. A set of 174 loci were analyzed, 120 of which were polymorphic. Genetic diversity was estimated at different geographic levels for the total population and between cities. Genetic diversity averaged 0.32. The largest gene flow was detected between two population from Cali (F_{ST} = 0.088; *Nm* = 2.6) and the lowest from the third population in this city (Univalle) and Popayán and Buenaventura (F_{ST} = 0.13; *Nm* = 1.6 and F_{ST} = 0.12; *Nm* = 1.8, respectively). The individuals' low mobility, the population' geographic separation, and possibly genetic drift have resulted in the population of this cockroach species having a significant degree of structuring, both between cities (Φst = 0.13; P < 0.001) and within them (Φst = 0.87; P < 0.001). These results suggest a high degree of subdivision within the *P. americana* population studied.

KEY WORDS: Genetic structure, population differentiation, urban pest

Periplaneta americana (L.) is considered a highly synanthropic cosmopolitan urban pest, which in tropical weather is capable of surviving and adapting to diverse environments including intra- and peridomiciliary (Cornwell 1968, Bell & Adiyodi 1981). It is not very clear how cockroach populations relate to each other within urban environments, and the dispersion methods are not well known what is important for establishing efficient control programs against this major urban pest. Genetic variability can be lost in fragmented or isolated population; and it is not completely clear how cockroach populations interrelate. It is considered that passive movements of cockroaches are commoner within cities than between them as they are facilitated by human activity. Following this line of thought, it is expected that a city's population is more homogenous than between distant locations.

On the other hand, a founder effect tends to be frequent in urban Blattidae population. It has been demonstrated that this and other factors such as selection, genetic drift and migration affect structure and genetic differentiation (Cloarec *et al* 1999). According to the authors, these population structure and genetic differentiation can be quantified by calculating several statistical parameters based on population polymorphism data, which can contribute information on the population's history. These parameters, together with genetic relations, phylogenetics and population dynamics, can be calculated from band patterns obtained through the use of molecular markers (Behura 2006).

Although *P. americana* is one of the best studied insect species at biological level, little is known about its genetic variability, which affects the dynamics and importance of factors as migration of active and passive individuals, selection pressure due to insecticides, bottleneck and founder effects in the population's structuring. Thus it was proposed to estimate the genetic variation and degree of genetic differentiation among and between *P. americana* population in three cities of southwestern Colombia through the use of AFLP (amplified fragment length polymorphism) molecular markers.

Material and Methods

Samples of *P. americana* were collected within the urban perimeter of three cities, located in southwestern Colombia: Santiago de Cali (3°26'14'' N, 76°31'21'' W, 991 masl, 24°C, rainfall 1473 mm), Buenaventura (3°53'36'' N, 77°4'11'' W, 57 masl, 25.4°C, rainfall 6980 mm) and Popayán (2°26'39'' N, 76°37'17'' W,1738 masl, 19°C, rainfall 2006 mm).

In each one of these cities, specimens were collected from two to three different areas within the urban perimeter. In the city of Santiago de Cali, two populations were obtained (Departamental and Ciudad Jardín neighborhoods). An additional population, kept in the Entomology labs of the Universidad del Valle (Univalle) for a little over 10 years,

was also analyzed.

In Buenaventura and Popayán, the work was done with a single population per city. The material was collected manually during the night (between 21:00h and 24:00h) in external areas such as front yards, water and light meters, and sewers. Approximately 40 adult individuals, both male and female, were collected from each site.

The samples were taken to the Univalle (Cali) entomology insectarium, where they were reproduced until obtaining sufficient individuals for performing several studies. Each of the population collected was bred following Bell (1981), with some modifications. For each colony petri dishes with dog food pellets and cotton saturated in water were provided. From these colonies, 14 tissue samples (one per female) were collected at random from each of the five populations.

DNA extraction. A strict protocol for extracting the DNA, recommended by Pat Roman's lab at the University of Toronto, modified by Coen *et al* (1982), and described in Black & DuTeau 1997) was followed. Concentrations and recommended quantities were standardized for DNA extraction from *P. americana*.

Coxa III was used from each specimen. Each sample was macerated separately with a Kontes grinder in a 1.5-ml microcentrifuge tube. To each of the macerated samples, 400 µl of extraction buffer (5 M NaCl, 0.5 M EDTA, 1 M Tris HCL, 20% SDS, 0.2 M sucrose) were added, and incubated at 65°C for 30 min. Afterwards, 140 µl of 8 M potassium acetate was added and incubated in ice for 30 min. The samples were centrifuged at 4ºC for 10 min at a speed of 15900 *g*, and the supernatant was transferred to another microtube. Then, 800 µl of cold absolute ethanol was added, and the DNA was left to precipitate overnight. Afterwards, it was centrifuged at 4°C for 20 min at 12230 *g*. This last step was repeated twice, and then the DNA was resuspended in 100 µl of TE (1M Tris HCL, pH 8.0 0.5M EDTA) and stored at -20°C.

Each sample was quantified in a fluorometer (TKO 100 Mini-Fluorometer, HOEFER Scientific Instruments), using a Bio Rad kit (catalog no. 170-2480), with Hoechst 33258, cow thyme DNA and 10x TEN buffer. The DNA pattern sample was used at a 100 ng/µl concentration.

AFLPs. Each DNA sample was diluted to 300 ng/µl. To perform the reactions, an analysis system I kit (for large genomes) by AFLP™ produced by INVITROGEN was used, following the producer's recommendations. Briefly, the components for this system with their respective quantities were as follows: Digestion was performed in 0.2-ml PCR tubes, to which were added 5 µl of 5X reaction buffer, 7.5 µl of sample DNA (300 ng/µl), 2 µl EcoRI/MseI and 10.5 µl of AFLP-grade distilled water. Samples were incubated at 37ºC for 2h, and then at 70ºC for 15 min, and lastly, temperature was stabilized at 4ºC.

Then 24 µl of adapter ligation solution and 1 µl of T4 DNA ligase were added to the 25 µl of digested product. The product was mixed at room temperature, left at 20ºC for 2h and then stored at -20ºC. Contrary to the kit's suggestion and given that a positive preamplification of the ligation products was not obtained during standardization of the technique, 1:10 dilutions of these products were not performed prior to preamplification.

During preamplification (PCR+1) of the ligation products, microcentrifuge tubes with 0.2-ml thin walls were used, in which 5 µl of DNA (digestion-ligation product), 40 µl of preamplification primer mix, 5 µl of 10X PCR + Mg buffer and 1 µl of *Taq* DNA polymerase were mixed. The amplification program consisted in setting the samples at 94ºC for 30 s, then at 56ºC for 60 s and at 72ºC for 60 s. This procedure was repeated for 20 cycles, and then the temperature was stabilized at 4ºC.

Preamplified samples were diluted to a 1:50 proportion. Then 147 µl of TE buffer was added to 1.5-ml eppendorf tubes, after which 3 µl of the preamplification samples were added to each one. This was enough for 30 selective amplifications 1.5-ml eppendorf tubes; the remaining solutions were stored at -20ºC. During selective amplification (PCR + 3), each EcoRI primer was diluted in distilled water (18 µl EcoRI primer, 32 µl distilled water). For each primer combination two reaction mixtures were performed in 1.5-ml microcentrifuge tubes. Mix 1 was composed of 5 µl of EcoRI primer and 45 µl of MseI primer; Mix 2, 79 µl of distilled water, 20 µl of 10X PCR + Mg buffer and 1 µl of *Taq* DNA polymerase.

Each AFLP (PCR+3) amplification was performed by making a new reaction mix in 0.2-ml tubes, mixing 10 µl of mix 2, 5 µl of mix 1, and 5 µl of preamplified and diluted (1:50) DNA sample. The amplification program for the samples was as follows: 94°C for 30 s, 65°C for 30 s, 72°C for 1 min for 1 cycle. Then 94°C for 30 s, 65°C for 30 s (lowering 0.7°C per cycle each time), 72°C for 1 min for 12 cycles. Lastly, there were 23 cycles at 94°C for 30 s, 56°C for 30 s and 72°C for 1 min.

The standardization process of this technique was carried out in the Molecular Biology lab of the Biology Department at Univalle. Both the preamplification and selective amplification were performed in a Perkin Elmer Applied Biosystems Gene Amp PCR System 970 thermocycler.

Gel electrophoresis. This was done in denaturing gels using 6% polyacrylamide and 3 M urea. Initially, a prerun of the gel was performed at 50ºC and ~100 W for about 1h (or long enough for the system to stabilize at 50ºC). Each sample was prepared as follows: First, 2 µl of loading buffer was added to 5 µl of sample; then these samples were denatured at 94ºC for 3 min and immediately stored in ice. They were quickly poured in each of the gel pits. Electrophoresis was performed with a 1X TBE buffer with a constant current (~80 W and approx. 1800 V) until the xylene cyanol front reached approximately 42 cm from the top.

Silver staining in polyacrylamide gels. Staining of the gels followed the procedure proposed by Ceron (2002) in which 50 x 38 cm trays were used. Glass with the gel was submerged in 3500 ml of fixation solution (10% acetic acid) for 20 min. Afterwards, it was washed with deionized water three times for 2 min, submerged in 3500 ml of staining solution for 30 min, and then washed with deionized water for 10 s and in 3500 ml of revealing solution for 3-5 min. Lastly, the gel was submerged in 3500 ml of fixation solution (10% acetic acid) for 5 min and washed with deionized water for 3 min. After the

staining process, the glass with the gel was left to dry overnight to be read and photographed the following morning.

Data analyses. Data on patterns of the AFLP bands in each DNA sample were coded in a binary data matrix (presence/absence). The polymorphic information content (PIC = 1-$\sum Pi^2$ - $\sum\sum 2Pi^2Pj^2$) was calculated in order to choose the two combinations for performing the tests. The TFPGA (Tools for Population Genetic Analyses) program, v. 1.3 (1997) was used to generate a distance matrix (1-S) from Nei and Lei's (1979) similitude indexes. Based on this matrix a descriptive analysis of the population was performed, calculating the allelic frequency and expected heterozygosity for each locus.

With the POPGENE 1.31 (1999) program, estimates were made of the allelic frequency for each locus, the effective allele number, the percentage of polymorphic loci and Shannon's index as a measure of genetic diversity and genetic distance and identity (Nei 1972, 1978). Average genetic diversity or expected heterozygosity was calculated based on the formula proposed by Nei (1973).

Additionally, a neutrality test was performed, using the Arlequin 3.0 (Excoffier *et al* 2005) program. Tajima's (1989) selective neutrality test was chosen, based on the infinite sites without recombination model, recommended for RFLP haplotypes. For the molecular variation analysis, the program WINAMOVA 1.55 (Excoffier 1993) was used, computing the molecular variation components within and between populations, fixation indexes (Phi_{st}), coancestry coefficients and heteroscedasticity index (Bartlett's statistic). Statistics F_{ST}, Theta, F_{ST} (Lynch) and number of effective migrants were calculated using the program RAPDFST 4.0.1 (Black 1997). The F_{ST} statistical was estimated based on the formula proposed by Wright (1931). To observe the relations between the studied population and individuals of *P. americana* graphically, dendrograms were done using UPGMA, based on Nei's (1972) genetic distances.

Results

Nineteen combinations of primers were evaluated for a total of 1083 loci, of which 79% were polymorphic. The polymorphic information contents (PIC) of the evaluated combinations ranged from 0.2 to 0.5. The AGG-CTT (PIC = 0.48, 88.4% polymorphic loci) and AAC-CAG (PIC = 0.42, 85.3% polymorphic loci) combinations were chosen for evaluation in the five *P. americana* populations studied.

From the two combinations evaluated, a total of 174 loci were obtained. The percentage of polymorphic loci was 80.46 (140 loci). According to what was established by Lynch & Milligan (1994), those loci with a frequency of one or two presences and/or one or two absences must be discarded. In this case the analyses were performed on the basis of 120 polymorphic loci. The population genetic analyses showed that the percentage of polymorphic loci per population was between 63.3% (Popayán) and 88.3% (Cali-Ciudad Jardín). The greatest genetic diversity (*h*) was found in the Cali-Ciudad Jardín population (0.33); the same was true for this population's Shannon's index (0.48). The population with the lowest genetic diversity and Shannon's index was Popayán (0.22 and 0.33, respectively) (Table 1).

With the neutrality test, probabilities greater than 0.05 (P = 0.80-0.89; SD 0.04) were obtained; therefore, the alleles of these *P. americana* population can be considered neutral and the punctual mutations, synonyms. Given the values obtained in the *D* statistics, (0.67-1.11; SD 0.17), it is safe to assume that the evaluated population have not suffered recent bottlenecks; but it is possible that there has been a genetic drift process. Four of the five *P. americana* populations studied had a unique allele, two of the populations from the city of Cali shared five private alleles and shared an additional allele with the Cali-Univalle population. Not only is the presence of this kind of allele important, but also their absence in some populations. Of the total alleles studied for the five *P. americana* populations, 3.33% were not found in the Popayán population, 2.5% of the alleles were absent in the Buenaventura population, and a similar percentage was absent in the Cali-Univalle population. The allelic frequencies for each population were greater than 0.05; therefore, their fixation is not recent.

Analysis of molecular variance (AMOVA) showed that most of the observed variation is among individuals within the population (Φ_{it} = 0.87, P < 0.0010). The rest of the variation can be attributed to the variation between population (Φ_{st} = 0.13, P < 0.0010) (Table 2). This matches with the population' high heterozygosity levels, indicating a significant level of differentiation among the *P. americana* population evaluated.

Table 1 Genetic analyses of five *Periplaneta americana* population for 120 AFLP loci.

Population	N	Size *na*[1]		*ne*[2]		*h*[3]		*I*[4]		Polymorphic loci	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD[5]	$\bar{x}$	SD	N	%
Cali-Univalle	14	1.70	0.46	1.45	0.39	0.26	0.20	0.38	0.28	85	70.8
Cali-Ciudad Jardín	14	1.88	0.32	1.57	0.35	0.33	0.17	0.48	0.23	106	88.3
Cali-Departamental	14	1.75	0.43	1.45	0.37	0.26	0.19	0.39	0.27	90	75.0
Popayán	14	1.63	0.49	1.39	0.40	0.22	0.21	0.33	0.29	76	63.3
Buenaventura	14	1.71	0.46	1.43	0.38	0.25	0.20	0.37	0.27	85	70.8
Total	70	2.00	0.00	1.54	0.32	0.32	0.15	0.48	0.19	120	100

[1]*na* = Observed number of alleles; [2]*ne* = Effective number of alleles (Kimura & Crow 1964); [3]*h* = Nei's (1973) gene diversity; [4]*I* = Shannon's Information Index (Lewontin 1972); [5]SD = Nei's (1978) standard deviation

Table 2 Analysis of molecular variance (AMOVA) of 70 *Periplaneta americana* DNA samples from five populations in southwestern Colombia, each represented by 14 individuals and 120 AFLP fragments.

Source of variation	df	SS	MS	Variance components	F	P
Among population	4	205.83	51.45	2.48 (13.0 %)	3.08	< 0.0001
Within population	65	1084.07	16.68	16.68 (87.0 %)		
Total	69	1289.90				

PHI statistics: $\Phi_{st} = 0.130$; $P < 0.0010$
Bartlett's heteroskedasticity index (1937): Variance heterogeneity among population: $Bp = 0.39664$ ($\chi 2$, df = 4, $P < 0.0009$)

The estimated distances between the evaluated population pairs were significant, which suggests nonrandom observed values of Φ_{st}. The population pair with the greatest difference was Cali-Univalle and Popayán (0.18) (Table 3). Bartlett's test to determine the variance among population' heterogeneity was highly significant ($P < 0.001$), indicating that the magnitude of the molecular variance expressed in each population was different from that of other population and that there is population substructuring as well.

An average F_{ST} of 0.160 ($P < 0.001$) was obtained for all populations, which represents a significant differentiation among them. This same trend was present in the other statistics calculated ($\Theta = 0.182$, F_{ST} Lynch & Milligan = 0.204) (Table 4). For the three calculated parameters, the standard deviation was high, confirming substructuring and a higher variance at an intrapopulation level.

The F_{ST} values were significant for each pair of populations. The Cali-Univalle population had the highest differentiation values against the Popayán ($F_{ST} = 0.13$) and Buenaventura ($F_{ST} = 0.12$) population. Accordingly, both population pairs had the lowest number of effective migrants. The average number of effective migrants (*Nm*) between the populations was 1.3, the highest number of effective migrants between two populations was in the city of Cali (2.6); however, a high number of migrants was also present, among some of the populations from the other cities evaluated, even among individuals from the Cali-Univalle population (kept in the lab) and Cali-Ciudad Jardín population (*Nm* = 2.3). These results confirm the origin of the parentals for the population kept in the lab (Cali-Univalle). Likewise, due to these individuals' isolation in the lab for over ten years, they constitute the most distant for the Popayán and Buenaventura population (Fig 1). The results for genetic distances and identities (Nei 1972, 1978) (Table 5) coincide with what was observed for the *F* statistics.

Analyses with the UPGMA cluster showed that only one of the Cali populations (Departamental) had more genetic similarity than Popayán. This group is also related to the Buenaventura and Cali-Ciudad Jardín populations (Fig 1).

Discussion

AFLP is a potentially useful technique in genetic studies of *P. americana* population as more than 100 polymorphic markers were achieved in this study with only two primer combinations. Parsons & Shaw (2001) found a similar number of fragments using this technique with four primer combinations in cricket population of *Laupala* in the Hawaiian islands. According to Nei (1978), with over 50 loci, it is possible to obtain a good estimate of the average heterozygosity in the evaluated population, which compensates for a small number of individuals evaluated per population.

The proportion of polymorphic loci (80.46%) and expected average heterozygosity (He = 0.32) found in *P. americana* population were high and above the overall mean, indicating a high level of polymorphism in this species. Extreme values of heterozygosity have also been observed in other insect species such as the Diptera *Drosophila bifasciata* Pomini (Drosophilidae) (Ho = 0.24) (Cariou *et al* 1990), *Anopheles nuneztovari* Gabaldon (Culicidae) (Ho = 0.34) (Posso *et al* 2003), *Aedes aegypti* L. (Culicidae) (Ho = 0.35) (Apostol *et al* 1996) and the Australian wood cockroach *Panesthia australis* Saussure (Blattodea: Blaberidae) (Ho = 0.80) (Runciman *et al* 2006), as well as in other arthropods belonging to the Araneae order (Steiner & Greenstone 1991). However, similar studies with allozymes in the German cockroach have shown very low levels of polymorphism, *Ho* = 0.059 (Cloarec *et al* 1999), ratifying the subestimation of genetic diversity with this kind of marker.

The relatively high heterozygosity values suggest that

Table 3 Distances Φ_{st} between pairs of *Periplaneta americana* populations, each represented by 14 individuals and 120 AFLP fragments. All distance values are highly significant ($P < 0.001$). No. of permutations = 1000.

Population	Cali (Univalle)	Cali (Ciudad Jardín)	Popayán	Buenaventura	Cali (Departamental)
Cali (Univalle)	-				
Cali (Ciudad Jardín)	0.13	-			
Popayán	0.18	0.14	-		
Buenaventura	0.14	0.14	0.15	-	
Cali (Departamental)	0.10	0.10	0.12	0.09	-

Table 4 F_{ST} statistics, theta and F_{ST} of Lynch & Milligan (1994) for each of the combinations between pairs of *Periplaneta americana* populations evaluated; and number of effective migrants (*Nm*), bootstrapping over loci and 1000 permutations (P = 0.001).

	F_{ST}		*Nm*	Theta		*Nm* (Theta)	F_{ST} (Lynch & Milligan)		*Nm* (Lynch & Milligan)
	$\bar{x}$	SD		$\bar{x}$	SD		$\bar{x}$	SD	
All populations	0.16	0.14	1.3	0.18	0.17	1.1	0.20	0.21	1.0
Cali-Univalle/Cali- Ciudad Jardín	0.10	0.14	2.3	0.16	0.20	1.3	0.15	0.19	1.4
Cali-Univalle/Popayán	0.13	0.20	1.6	0.24	0.24	0.8	0.23	0.24	0.8
Cali-Univalle/ Buenaventura	0.12	0.17	1.8	0.22	0.23	0.9	0.21	0.21	0.9
Cali-Univalle)/Cali-Departamental	0.11	0.15	2.1	0.19	0.21	1.1	0.18	0.20	1.1
Cali-Ciudad Jardín/Popayán	0.11	0.14	2.0	0.18	0.20	1.1	0.17	0.18	1.2
Cali-Ciudad Jardín/Buenaventura	0.10	0.13	2.2	0.17	0.19	1.2	0.16	0.17	1.3
Cali-Ciudad Jardín/ Cali-Departamental	0.09	0.13	2.6	0.14	0.18	1.5	0.13	0.17	1.7
Popayán-Buenaventura	0.11	0.14	2.1	0.19	0.20	1.1	0.18	0.18	1.2
Popayán/Cali- Departamental	0.09	0.13	2.4	0.16	0.18	1.3	0.15	0.17	1.5
Buenaventura/Cali-Departamental	0.09	0.12	2.4	0.16	0.18	1.3	0.15	0.17	1.4

these *P. americana* populations have not experienced recent bottlenecks; that is, the effective size of the *P. americana* population in the three cities has not been small. Heterogeneous habitats within urban ecosystems could protect cockroach population from diverse selection pressures. Among the most preferred nesting places in the urban environment are those located in the peridomiciliary, which is characterized by great stability and high biotic diversity (Schal & Hamilton 1990).

The source of the populations evaluated during this study was the peridomiciliary, especially in sites such as sewer boxes, water and light meters, and front yards. Despite not evaluating individuals captured in the intradomiciliary, it is evident that within human habitats, it is also possible to find an environment suitable for maintaining *P. americana* population, maintaining a high genetic diversity given that in this environment, resources and environmental stability interact in such a way that guarantee reproductive success. According to Schal & Hamilton (1990), these factors favor the establishment of large population of dominant species and small population of associated species including symbionts and natural enemies. The peridomiciliary is a rich location, where *P. americana* population maintain large numbers with high genetic diversity, which can be observed in the AMOVA results, which showed significant variance between individuals within the population (87.04%). It is probable

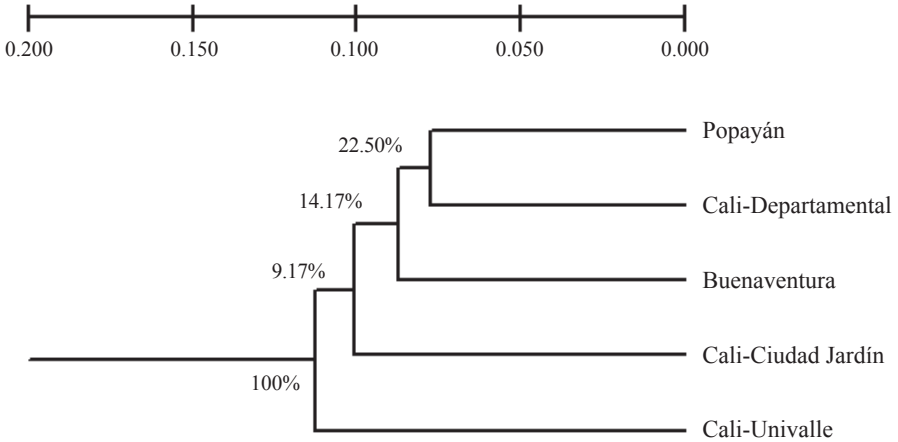

Fig 1 Dendrogram with the phylogenetic relations among the five *Periplaneta americana* populations from southwestern Colombia evaluated using the UPGMA cluster model (Nei 1972).

Table 5 Comparisons between pairs of *Periplaneta americana* populations (genetic distances and identities, Nei 1978). All distances and identity values are highly significant (P < 0.001). No. of permutations = 1000.

Population	Cali-Univalle	Cali-Ciudad Jardín	Popayán	Buenaventura	Cali-Departamental
Cali-Univalle	****	0.916	0.895	0.898	0.914
Cali-Ciudad Jardín	0.088	****	0.913	0.913	0.927
Popayán	0.111	0.091	****	0.925	0.937
Buenaventura	0.107	0.091	0.078	****	0.93
Cali-Departamental	0.089	0.075	0.065	0.072	****

Genetic identity (above diagonal) and genetic distance (below diagonal)

that populations in the intradomiciliary have similar traits; however, it is necessary to perform studies to compare the genetic variability of population close to *P. americana* sourced in the peridomiciliary and the intradomiciliary.

The founder effect seems to have had no influence on the degree of genetic diversity found in these populations. One possible explanation could be the fact that this species has little intraspecific competition. Studies done by Boyer & Rivault (2004) showed that resident individuals and conspecific intruders mix homogenously in the nest. Additionally, thanks to the aggregation pheromone present in this species, migrant individuals tend to find conspecifics to establish with them in the nest. This possibly helps the new residents to have the same survival and mating probability as the rest. This behavior mostly avoids the founder effect and therefore a reduction of genetic diversity within the population.

In conjunction with the foregoing, genetic diversity could be maintained by a large and constant gene flow between population through natural dispersion (Reiter *et al* 1995) and human-assisted migration via passive transport of individuals and oothecae (Chadee 1990). Through either of these modes, the cockroach populations have the possibility of exchanging genetic material among them. This was evident when observing the number of effective migrants among the *P. americana* population evaluated. Besides, *P. americana* tends to move over short distances in the peridomiciliary and the movement from these areas towards the intradomiciliary is not quite clear (Schal & Hamilton 1990). So, it can be supposed that the passive transport of individuals is one of the most important factors in the dispersion of individuals over great distances. Two of the populations evaluated in the city of Cali had the highest number of effective migrants (2.6), which could occur given that the movement and shipping of parcels is commoner within a city that between cities, even if they are relatively close to each other. The Cali-Univalle population had a higher number of effective migrants with the other two populations located in Cali than the Popayán and Buenaventura populations. This is due to the source of the parentals from which the colony was founded 10 years ago: sewer boxes located on campus of the Universidad del Valle and close to the Ciudad Jardín neighborhood, giving a higher number of migrants.

According to the results obtained with the F_{ST} statistic, the greatest markers of genetic differentiation were present in the population of Cali-Univalle)/Popayán (F_{ST} = 0.13) and Cali-Univalle/Buenaventura (F_{ST} = 0.12). This could be explained by considering the source of the Cali-Univalle population (see above), where not only the geographic distance between cities would be influential, but also the time that this colony has been kept isolated in the lab. The high genetic differentiation between the populations of Buenaventura and Popayán and the relative closeness of the former with the population in the city of Cali is due to the fact that Buenaventura, being the principal sea port on the Pacific, receives a large percentage of the merchandise taken into the countryside. It is quite probable that individuals, either adults or nymphs, and even oothecae are being transported in the different merchandise that leaves the port. This merchandise is generally taken first into the city of Cali, where it is distributed towards its final destinations including Popayán, which is the capital of a province next to the Valle del Cauca.

This differentiation between the *P. americana* populations evaluated can also be observed in the presence of private alleles. Although 72% of the total alleles studied were present in the five evaluated populations, each of them had a unique allele (except for the population from Popayán). Of the alleles studied, 4.2% were exclusive to the city of Cali, without including the Cali-Univalle population; however, a unique allele was found for all three of the Cali population. This confirms that population within the same city have higher genetic exchange, in this case thanks to human-assisted movements. In the population from Popayán, 3.3% of the alleles present in the other population studied were not found, what suggests that the genetic exchange between Popayán and the other cities is low. At this level, the differentiation between the three evaluated cities and the Cali-Univalle population should be highlighted due to the isolation time in the lab of the latter as three of the alleles shared by the population from the cities of Cali, Buenaventura and Popayán were not found in the Univalle population.

It must be taken in consideration, that some of the private alleles found in the *P. americana* population evaluated were probably fixed by genetic drift due to the substitution processes in which a mutation is fixed. This was evident when observing the lack of some bands in 100% of the individuals from the population evaluated. For example, none of the individuals of the Popayán population had amplification of four bands, which were present in the other population, and which were therefore considered as private alleles for this population. The same occurred for three missing alleles in 100% of the individuals from Buenaventura and for three alleles in the Cali-Univalle population. When the only acting evolution mechanism is genetic drift, the substitution rate equals the mutation rate, independent of population size, as more mutations are

produced in large population; but in a large population every new mutation has a lower chance to be fixed through drift. If genetic drift is acting, large population produce and maintain more genetic variation than smaller population; however, all the population, regardless of their size, accumulate substitutes at the same rate (Freeman & Herron 2002).

Although this is the first study of this kind with *P. americana* worldwide, similar studies have been done with other important cockroach species such as the urban pest *Blatella* germanica L. (Blattodea: Blaberidae). Cloarec *et al* (1999) used enzymatic markers to study the genetic structure of 31 of this species population originating from two French cities. A significant genetic differentiation was observed both among and between the population of each of the cities; however, a significant difference between cities was not found. Although there was evident substructuring at a local level, differentiation on a larger geographic scale could not be confirmed. These results oppose those found in this research with *P. americana*, which found a significant genetic difference between cities, besides substructuring at a local level.

These two cockroach species, considered as the most important urban pests worldwide and known for their great capacity for adaptation, can face similar ecological processes such as succession and replacement of species at earlier phases, both in the intra- and peridomiciliary (secondary ecological communities). Within these environments, they experience competition, predation or minimal habitat modifications, which helps them become the dominant species within these communities (Schal & Hamilton 1990). These and other shared ecological features such as being "r" strategists are not enough to make a generalization of all the urban pest cockroach species. This confirms that other factors such as the inherent behavior of each species, along with selection, genetic drift, migration and founder effects, are affecting the structure and genetic differentiation not only of different species, but of population between and within locations.

According to the proposed hypothesis, the structuring and genetic differentiation of *P. americana* population in southwestern Colombia are possibly related to the low mobility of individuals outside their colony, which is limited by geographic and ecological barriers. However, genetic drift and tolerance of individuals hailing from other population help to keep the genetic variability of these cockroach populations.

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