Fine Scale Analysis of Genetic Structure in an Argentine Population of *Anastrepha fraterculus* (Wied.) with SSR markers.

Ángeles Iriel Rodriguez[1], Laura Inés Ferreyra[1], Silvia Beatriz Lanzavecchia[2], Paula Valeria Gómez Cendra[1], Juan César Vilardi[1]

[1] Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Inst. Ecología, Genética y Evolución (IEGEBA). Fac. Cs Exactas y Naturales. Dept Ecología, Genética y Evolución. Genética de Poblaciones Aplicada (GPA) (C.P. 1428) Buenos Aires, Argentina. [2] Inst. de Genética 'Ewald A. Favret', Instituto Nacional de Tecnología Agropecuaria, Hurlingham, Argentina

Introduction

The South American fruit fly *Anastrepha fraterculus* causes significant damage to fruit and vegetable crops. Information about dispersal and oviposition behavior in the wild is relevant to integrated pest management programs.

These questions may be approached by population structure analysis using molecular markers.

Our objective was analyzing the adaptive strategy and population structure of this species in a natural population from Argentina using microsatellite (SSR) markers.

Methods

Sampling

More than 200 individuals were recovered from about 150 infested fruits from 10 guava trees in an orchard near Horco Molle, Tucumán, Argentina. In order to conduct hierarchical structure analysis the sample was restricted to get several fruits per tree yielding several individuals each. The final number of genotyped individuals was 65 (18 fruits from 9 trees).

DNA Extraction

It was performed according to the protocol specified by Baruffi et al. 1995 (Heredity 74: 425-437)

SSR

Six SSR loci where amplified in PCR conditions: One cycle at 95°C (2 min), 30 cycles at 95°C (30 s), 58° C (30 s) and 72°C (30 s). Final elongation at 72°C (10 min). Performed in a Veriti Thermal Cycler, Applied Biosystem.

PCR Analysis

Fragments were run in an automatic sequencer 3500xl Genetic Analyzer, Applied Biosystems, and processed by Gene Marker v2.4.

Statistical Analysis

Genetic variability was estimated by several indices (Tables 1 and 2). Population structure was studied by Wright's *F*-statistics and analysis of molecular variance (AMOVA) considering three different hierarchical levels (trees/fruits/individuals). Reynold's genetic distances were estimated for UPGMA tree and cluster analysis. Genotype accumulation curve was also estimated. All analyses were performed with the packages *hierfstat*, *poppr*, *ade4* and *ape* of the software **R**.

Results and Discussion

Genetic variability and structure population analyses

Locus	*Ho*	*Hs*	*Ht*	*FST*	*FIS*
D105	0.346	0.448	0.498	0.100	0.227
A115	0.400	0.688	0.782	0.120	0.419
A7	0.581	0.744	0.764	0.026	0.219
A120	0.676	0.788	0.870	0.094	0.143
C103	0.803	0.806	0.842	0.043	0.004
A10	0.641	0.608	0.667	0.089	-0.054
Overall	0.574	0.680	0.742	0.077	0.156

Table 1. Genetic variability and population structure parameters per locus. All loci were highly polymorphic according to observed (*Ho*), expected (*Hs*), total heterozygosity (*Ht*), and number of alleles (*n*). Wright's F_{ST} indicated that about 8% of variation occurs among fruits and average F_{IS} is positive suggesting heterozygosity excess within fruits

Fruit	*H*	*Hs*	$\bar{f}_D$	Fruit	*H*	*Hs*	$\bar{f}_D$
10--2	1.39	0.462	-0.174	05--07	1.61	0.670	-0.013
02--01	1.61	0.667	-0.144	06--09	1.61	0.751	**0.226***
02--05	1.61	0.640	-0.013	07--03	1.61	0.754	0.008
03--12	1.61	0.614	-0.050	07--09	1.61	0.641	0.147
04--02	0.00	0.500	---	08--01	1.61	0.528	0.129
04--03	1.61	0.654	**0.370***	08--03	1.79	0.704	0.014
05--03	1.61	0.744	-0.064	09--04	1.61	0.707	0.049

Table 2: Genetic variability and population structure per fruit. Shannon-Weiner Diversity index (*H*), Expected heterozygosity (*Hs*), and Gametic disequilibrium ($\bar{f}_D$). Variability within fruits was high and the different loci are mostly independent as $\bar{f}_D$ in most cases is non significant. (*significant values)

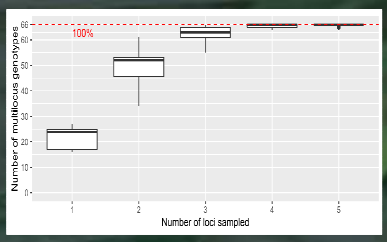

Figure 1: Genotype accumulation curve. Due to the high variability of the SSR loci the multilocus genotype of only 4-5 loci allows to discriminate 100% of individuals.

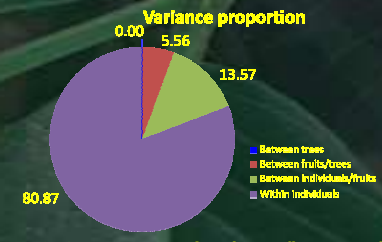

Figure 2: Components of variance (in proportions) estimated by AMOVA. The highest part of genetic variation occurs within individuals (*P*= 0.001), the remaining variation was observed between individuals within fruits (*P*= 0.001) and between fruit within trees (*P*=0.004). Variation among trees was non significant.

Cluster analysis and Minimum Spanning Tree

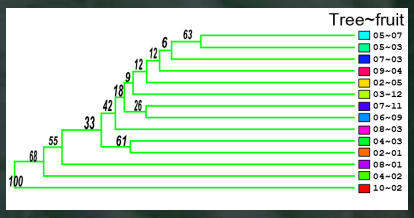

Figure 3: UPGMA tree based on Reynold's distance. Fruits were not grouped per tree. Boostrap support is represented by numbers over nodes. "Tree~fruit" code: the first number identifies the tree and the second the fruit.

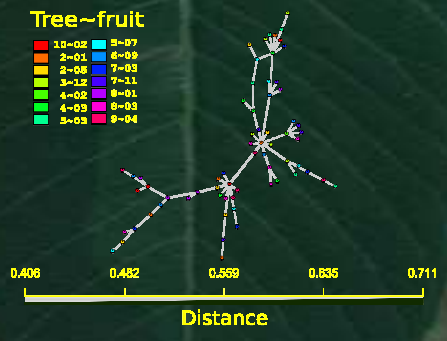

Figure 4: Minimum spanning tree based on Reynold's genetic distance. Individuals were not grouped per fruit or tree. Colors represent fruits and dots indicate individuals collected from them. The edge width is inversely proportional to the distance.

Conclusions

- The SSR markers used allowed a fine scale genetic population structure and diversity analysis
- All the performed analyses suggest that there would be no fidelity in female oviposition behavior to the same fruit and each fruit would be colonized by several females.
- Although still preliminary, the results suggest that ovipositing females are able to disperse significantly among trees throughout the orchard.