Population genetics of two neotropical *Drosophila saltans* group species.

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Introduction

The *Drosophila saltans* group is a member of the subgenus *Sophophora* (Sturtevant, 1942). All 16 species are found in the tropics, although the ranges of most have not been studied extensively (Markow and O'Grady, 2006). The group originated from a common ancestor in tropical North America (Throckmorton, 1975).

While phylogenetic relationships of the *saltans* group have been established using molecular tools (O'Grady *et al.*, 1998), the group as a whole, including population genetics and basic biology of many species, remains poorly understood. Here we present the first population genetic data for two *saltans* species, *Drosophila emarginata* and *Drosophila sturtevanti*.

Materials and Methods

Samples were collected from Pipeline Road in Gamboa, Panama between August 25th and September 8th, 2009. Bottle traps (Markow and O'Grady, 2005) were partially filled with a fermented banana, yeast, and water mixture. Flies were aspirated from the baits and held in banana food vials until sorted by species. The sorted flies were preserved in 95% ethanol and transported to UCSD. We extracted DNA from *D. sturtevanti* and *D. emarginata* using DNeasy kits (Qiagen). We used PCR to amplify the mitochondrial Cytochrome Oxidase I (COI) region for each sample. Samples were then sequenced and BLASTed to sequences in Genbank to verify species identification.

For each species, we used dnasp to calculate the following population genetic statistics: number of haplotypes observed, base pairs, polymorphic sites, haplotype diversity (*h*), and nucleotide diversity (π_2).

A haplotype network was constructed using TCS and dnasp. We were able to include data accessed through Genbank of a limited number of previously submitted COI sequences from both species.

Results and Discussion

The population statistics from the sequenced flies are shown in Table 1. *Drosophila sturtevanti* was more diverse than *D. emarginata* in haplotypes, polymorphic sites, and haplotype diversity. *Drosophila sturtevanti* also showed greater nucleotide diversity (0.0012 ± 0.0006) than *D. emarginata* (0.0003 ± 0.00004). The low haplotype number and low number of polymorphic sites in the saltans species could be indicative of small population size.

Both of these species show a relatively low level of genetic diversity. Haplotype diversity and nucleotide diversity values are comparable to other species such as *D. nigrospiracula*, a

cactophilic species found in the Sonoran Desert that shows low diversity compared to other sympatric species (Hurtado *et al.*, 2004). In contrast, a recent study done on *D. suzukii*, an invasive species from Asia, revealed five haplotypes in a sample of eight individuals (Carvajal, 2010).

Table 1. Summary table of haplotypes, polymorphic sites, haplotype diversity (*h*), and nucleotide diversity (π_2).

Species	N	Haplotypes Observed	bp	Polymorphic Sites	*h*	π_2
D. emarginata	19	3	616	2	0.205 ± 0.0273	0.0003 ± 0.00004
D. sturtevanti	29	4	605	4	0.406 ± 0.0102	0.0012 ± 0.0006

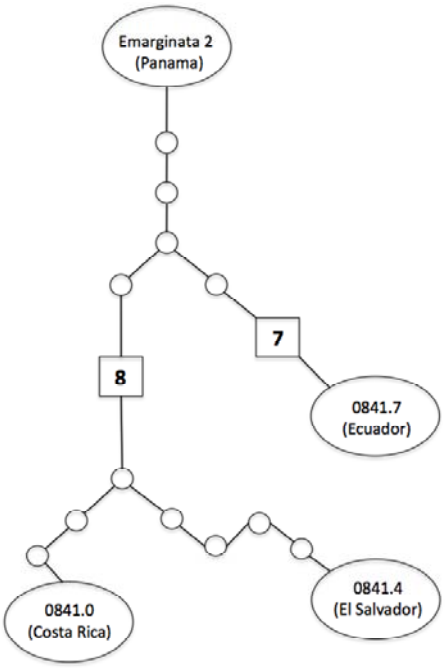

Figure 1a

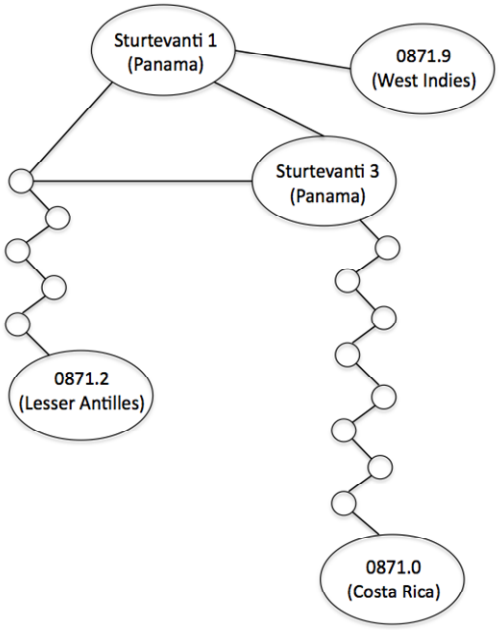

Figure 1b

Figure 1. Haplotype networks for both *D. emarginata* (Figure 1a) and *D. sturtevanti* (Figure 1b). Each circle represents a single nucleotide change. Number values in squares indicate the number of changes in that region. Because sequences from genbank only contained 305 base pairs, sequences from this study were trimmed to the same size, thereby eliminating some haplotypes from the analysis.

The haplotype networks for both species show considerable genetic separation. While the *D. sturtevanti* West Indies haplotype was similar to a haplotype from Panama, all others contain greater than five base pair changes between locations. Haplotypes from *D. emarginata* had more changes from location to location than *D. sturtevanti*.

While their geographic range is not well documented, both species have been collected in areas other than the isthmus region of Central America. Recent maps of the *saltans* species range show that both *D. sturtevanti* and *D. emarginata* are distributed from southern Mexico into Colombia and Brazil, with *D. sturtevanti* also in the Caribbean and *D. emarginata* into Peru (Markow and

O'Grady, 2006). Thus, data from both our population genetic analysis and haplotype networks support the notion that regional populations may be isolated from each other by environmental barriers. Sampling additional parts of the species range would reveal whether low mt COI variability is characteristic of these species as well as whether differentiation among regions exists.

Table 2. Table of sequences used for population genetic summary and haplotype network.

Species and/or line #	n	Collection Location	Genbank accession #
D. emarginata			
Emarginata 1	1	Gamboa, Panama	HQ696949
Emarginata 2	17	Gamboa, Panama	HQ696950
Emarginata 3	1	Gamboa, Panama	HQ696951
14042-0841.0	N/A	Turrialba, Costa Rica	AF045108
14042-0841.4	N/A	La Palma, El Salvador	AF045110
14042-0841.7	N/A	Quito, Ecuador	AF045109
D. sturtevanti			
Sturtevanti 1	22	Gamboa, Panama	HQ689648
Sturtevanti 2	5	Gamboa, Panama	HQ689649
Sturtevanti 3	1	Gamboa, Panama	HQ689650
Sturtevanti 4	1	Gamboa, Panama	HQ689651
14043-0871.0	N/A	Turrialba, Costa Rica	AF045098
14043-0871.2	N/A	Volcan, Soufriere, Lesser Antilles	AF045099
14043-0871.9	N/A	Martinique, West Indies	AF045100

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References: Carvajal, J.I., 2010, Dros. Inf. Serv. 93: 67-68; Hurtado, L.A, T. Erez, S. Castrezana, and T.A. Markow 2004, Mol. Eco. 13: 1365-1375; O'Grady, P.M., J.B. Clark, and M.G. Kidwell 1998, Mol. Biol. Evol. 15: 656-664; Markow, T.A., and P.M. O'Grady 2006, Drosophila: *A Guide to Species Identification and Use*. London: Elsevier Inc; Sturtevant, A.H., 1942, Univ. Tex. Publ. 4213: 6-51; Throckmorton, L.H., 1975, In: *Handbook of Genetics, Vol. 3. Invertebrates of Genetic Interest*. (King, R.C., ed.). Plenum Press, New York, pp. 421-469.

Preliminary studies on isolation and characterization of major glue protein genes in *Drosophila nasuta nasuta*.

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Abstract: The *Drosophila nasuta* salivary gland secretion protein gene/s are coordinately expressed in salivary glands of third instar larvae. The secretion consists of glycoproteins that could