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Chromosome-site interaction in the South American fruit fly *Anastrepha fraterculus* (Wied.)

Abstract

The present paper aims to further analyze and explain which is the significance of genetic variation in the so-called *Anastrepha fraterculus* complex and to solve if chromosomal vari-ants in *Anastrepha fraterculus* are associated to geographic variation. Our hypothesis are: I) chromosomal variants are not randomly distributed in the South American fruit fly populations studied. II) Chromosomal variants are not reproductive isolation markers. We sampled guava fruits from Argentina, Uruguay and Brazil during at least two years, to recover Anastrepha larvae as well as adult flies. The latter were single pair mated to form laboratory strains. We studied the chromosomal pattern of 879 larvae from wild populations and derived laboratory strains. Sexual chromosome variants were associated to different strains. Banding patterns were obtained with routine and molecular cytogenetics. Strains from the most distant localities were used in crossings. We computed a log lineal analysis of the data set in order to test the hypothesis of inertia and to get probabilistic estimates of relevant parameters associated with chromosome variation. We used a test of hypothesis to determine the existence of statistically significant associations between karyotypic frequencies relative to sex chromosome variants and the natural populations. With respect to hypothesis I, analyses showed ten sexual chromosome variants [(X1, X2, X3, X4) and (Y1, Y2, Y3, Y4, Y5, Y6)] and highly significant statistical chromosome site interaction, i.e. significant differentiation between observed data and those merely expected from random association of chromosome types with localities (inertia). When large samples from a given population were available, eight out of ten variants were found. With respect to hypothesis II, we could detect 28 different sexual karyotypes out of 34 possible combinations and it seems that no chromosome variant operates as a reproductive isolation marker. Our evidence is consistent with our previous suggestions, demonstrating that – within the regions studied- *A. fraterculus* is a single polymorphic species.

Keywords: *Anastrepha fraterculus*, population structure, polymorphisms, taxonomic status, log lineal analysis, chromosome x site interaction

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Introduction

The South American fruit fly *Anastrepha fraterculus* (Wied.) has a host range that encom-passes the fruits of a wide variety of cultivated plants. It shows preference for Psidium guayaba and is distributed into temperate to subtropical regions. In Argentina, *A. fraterculus* is the only economically important species within the genera Anastrepha. There is a hypothesis which states that this genus includes more than one biological species, proposing the existence of a complex of cryptic species. The confusion has been generated for several reasons.

i. Systematic studies have not found notable sexual differences and the identification of the species is mainly based on the apex morphology of the female ovipositor.[1]

ii. Morphological variation was described throughout its geographical range.[2]

iii. High karyotypic, biochemical and molecular variations are present within and among different geographic populations of the insect.[3–14]

iv. Due to laboratory rearing problems many authors[8,15] postulate that polymorphisms are marking either different species or that they are going through an isolation process within what may be termed the *Anastrepha fraterculus* complex previously proposed by Stone.[2]

v. Scarcity of managed crossings, as a consequence of laboratory rearing problems.[16]

According to Norrbom[17] the fraterculus species complex is composed by 17 groups of sibling species one of them being the fraterculus group involving 27 sibling species. Zucchi[18] had fused in synonymy of A. fraterculus, 17 species of the fraterculus group.

Mexican populations

Although we have not studied Mexican populations we have summarized the cytotaxonomic study made by Bush[4] on several fruit infesting Anastrepha in Mexico. The purpose was to observe if chromosome morphology would be of any use in identifying larvae. This author described the kayotypes of nine Anastrepha species but he could not find differences between the karyotypes of *A. fraterculus*, *A. distincta* and *A. mombinpraeoptans*.

Brazilian populations

Some authors[3,7] studied the karyotype of Bra-zilian populations of the insect. Solferini & Morgante[7] found karyotypic variation among

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populations, suggesting that two of the karyotypes belong to different cryptic spe-cies, named *A. fraterculus* and *A. sororcula* Morgante et al.[6] Selivon et al.,[13] studied two Brazilian geographic populations distinguishable through sexual chromosome morphology named A. sp 1 and A. sp 2; crossings among them rendered fertile offspring. Later on, the authors report four cryptic species named Anastrepha sp.1 aff. fraterculus, A. sp.2 aff. fraterculus, A. sp.3 aff. fraterculus and A. sp.4 aff. fraterculus (so named A. sp 1, A. sp 2, A. sp 3, A. sp 4.[13,14]

Argentinian and Uruguayan populations

Karyotypical studies in Argentine populations revealed sexual and autosomal diversity among and within regional samples and describe "fraterculus Argentina 1" (from now on fArg 1) as the most frequent karyotype present in all of them.[19–21] Different polymorphisms were found within the Montecarlo population (Misiones Province), and that of the X-chromosome was distinct in relation to its X1 and X2 variants.[20] The X1-chromosome was distinctive with an fArg 1 karyotype. The X2 chromosome as well as other sexual chromosomal variants of the X- and Y- were described for this population and for other Argentine populations as well. These variants – characterized through molecular banding techniques and through in situ hybridization using rDNA probes- were found in different combinations in the wild. As the laboratory strains carrying each one variant were reciprocally crossed, showed not to be reproductively isolated.[20,21] The main characteristic we focused on was the statistic prediction of each chromosomal variant frequency within each population, the emphasis of the present work being on the detection of isolation barriers associated to sexual karyotypes.

Materials and methods

Data studied

We sampled guava fruits from Argentina, Uruguay and Brazil during at least two years, to recover Anastrepha larvae as well as adult flies. The latter were randomly single pair mated to form laboratory strains. We studied the chromosomal pattern of 879 larvae from wild populations and derived laboratory strains. Sexual chromosome variants were isolated within different strains. Banding patterns were obtained with routine and molecular cytogenetics[22–24] and assisted to recognize different sexual chromosomal variants and karyotypes.

Sampling from Argentinian, Brazilian and Uruguayan populations

The number of specimens studied in each Argentinian population was the following: 131 from Buenos Aires province [BA] (Lat 34º36'14'' S; Long 58º22' 54'' W), 281 from Misiones province [MIS] (Lat 26º56' S; Long 54º24' W) and 164 from Tucumán province [TUC] (Lat 27º12' S; 65º35' W); a sample of 275 specimens from one site in Brazil [BRAZ] (Lat 31°45' S; Long 52°20' W), and 28 specimens from one location in Uruguay [URUG] (Lat 34°21′ 25″ S; Long 56°42′ 06″ W).

Reciprocal crossings

Strains from the most distant localities [BA] and [BRAZ], [BA] and [MIS] were crossed.

Statistical analysis

In order to investigate the possible existence of inertia in the data matrix, i.e., unexpected high or low observed frequencies in Location×Karyotype combination cells, and to get an estimation of such parameters, we constructed a Log Linear Additive Model[25] and tested the fitting of the data to it. The statistical specification of the model is presented in the Appendix.

We tested the null hypothesis (H0:μCS(ij)=0) in order to analyze statistically significant associations between the karyotypic frequencies of each particular sexual chromosome variant and the natural populations. Moreover, we obtained a maximum likelihood (ML) estima-tion of the interaction parameters.

Results

Chromosomal frequencies sampled from all 5 localities showed ten sexual chromosome variants (X1, X2, X3, X4 and Y1, Y2, Y3, Y4, Y5, Y6) and highly significant statistical chromo-some×site interaction, i.e. significant differentiation between observed data and those merely expected from random association of chromosome types with localities (inertia). The frequencies of chromosomes are presented in Table 1, and the corresponding single-term test results are shown in Table 2. Significant chromosome-site associations are shown in Table 3.

Table 1 Chromosome frequencies across sites

		Chromosome Variant										
		X1	X2	X3	X4	Y1	Y2	Y3	Y4	Y5	Y6	Total
Site	BA	96	0	1	1	5	3	0	0	25	0	131
	MIS	124	78	5	6	15	6	0	0	32	15	281
	TUC	108	15	0	0	31	0	0	0	10	0	164
	BRAZ	148	58	0	0	33	9	8	9	10	0	275
	URUG	10	7	0	4	3	0	4	0	0	0	28
	Total	486	158	6	11	87	18	12	9	77	15	879

Table 2 Single-term tests for the chromosome-site data. "D. of F." are Degrees of Freedom

Effect	D. of F.	Marginal c2	p-level
SITE (S)	4	294.74	< 0.000001
CHROMOSOME (C)	9	1500	< 0.000001
C ´ S Interaction	36	236.52	< 0.000001

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Table 3 Estimates and hypothesis testing of model parameters (only tests statistically significant at 5% level are shown)

Effect	Model Term	Percent Count	Effect (u)	St. Error	z-value
	BA – X1	10.67	0.6975	0.2931	2.38
	BA – X2	0.06	-2.7776	1.0526	-2.64
	BA – Y5	2.82	1.5072	0.4098	3.68
	BRAZ – X2	6.47	0.9154	0.3688	2.48
	BRAZ – Y3	0.94	1.1724	0.562	2.09
	BRAZ – Y4	1.05	1.7009	0.6053	2.81
C ´ S	MIS – X2	8.68	0.9378	0.3508	2.67
Interaction	MIS – Y1	1.71	-0.7175	0.3093	-2.32
	MIS – Y6	1.71	1.8208	0.5785	3.15
	TUC – X1	12	0.7881	0.3153	2.5
	TUC – Y1	3.48	1.3059	0.3537	3.69
	URUG – X1	1.16	-1.1564	0.3651	-3.17
	URUG – X4	0.5	1.8038	0.5928	3.04
	URUG – Y3	0.5	1.9699	0.6269	3.14

When large samples from a given population were available, eight out of ten variants were found, as evidenced for [MIS] population. The chromosome composition of *A. fraterculus* populations varied among localities. Within the best sampled populations ([TUC], [MIS], [BRAS]) we detected – through test of hy-pothesis- positive and negative associations between localities and some chromosomal variants. Chi square tests allowed us to reject the null hypothesis of no association, i.e. that these three populations are significantly different with respect to Y-chromosome variants distributions ($\chi 2$=86.02; p<0.0001) and with respect to X-chromosome variants distributions ($\chi 2$=43.57;p <0.0001).

As may be clearly seen in Table 2, data analysis demonstrated very highly significant differences in frequencies of insects both, among sites and among chromosome types, but there is also a very strong interaction effect between them. This is well illustrated in Figure 1 where it may be seen that the lines cross each other several times. The kind of interaction which is a rather qualitative one, i.e., a case of interaction where there is heterogeneity in the magnitudes of an effect and the effect has different sign in all categories.[26] Figure 1 shows the site profiles. Furthermore, not all the individual effects resulted statisti-cally significant (at least, at 1% level), i.e., resulting in rejection of the null hypothesis H0: ucs(jj) = 0. Table 3, shows the statistically significant parameters according to the Wald ststistics.[27]

With respect to hypothesis II, among the 34 possible existing sexual karyotypes (24 for males and 10 for females), we observed 28 different combinations (Figure 2) not only in the wild but also in the laboratory strains which rendered offspring throughout more than 60 generations. It seems that no chromosome variant operates an isolation marker. Our evi-dence is consistent with the fact that variants recombine. Crosses between the most distant populations [BA] and [BRAZ], [BA] and [MIS] produced fertile offspring, suggesting that *A. fraterculus* is a single polymorphic species.

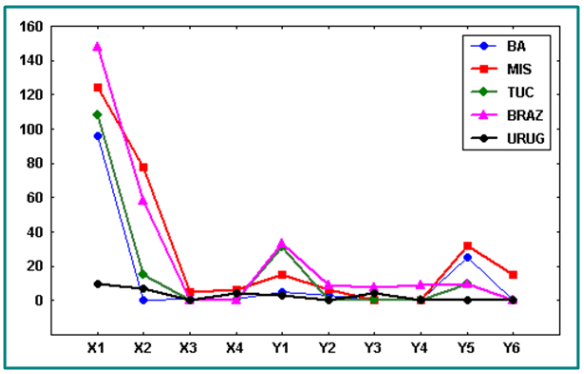

Figure 1 Chromosome frequencies across sites.

Males				Females						
X_1Y_1	X2Y1	X3Y1	X4Y1	X1X1		X2X2		X3X3		X4X4
X_1Y_2	X2Y2	X3Y2	X4Y2		X1X2		X2X3		X3X4	
X_1Y_3	X2Y3	X3Y3	X4Y3		X1X3		X2X4			
X1Y4	X2Y4	X3Y4	X4Y4		X1X4					
X1Y5	X2Y5	X3Y5	X4Y5							
X1Y6	X2Y6	X3Y6	X4Y6							

Figure 2 Possible sexual karyotypes for all 10 sexual chromosomal variants. Karyotypes not found are in bold.

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Discussion

In the present research, we used a data matrix with a total number of 879 larvae, i.e., an average of 207.4 larvae per population. The X1 chromosome is, by far, the most abundant one in all sites. Its frequencies are in-creased in [BA] and [TUC] but decreased in [URUG] due to interaction effects (Table 3). The X2 variant is also a very frequent chromosome although not as predominant as the X1: it was not found in [BA] (Table 1). The (ucs(jj)) was estimated in -2.7776 indicating a de-creased frequency in the order of hundreds with respect to the average frequency) and it had a statistically significant increased frequency in [BRAZ] and [MIS]. Chromosomes X3 and X4 are the rarest. Chromosome X3 was found only in [BA] and [MIS] and did not show any significant interaction. Chromosome X4 was found in [BA], [MIS] and [URUG]; it showed a positive significant interaction effect in [URUG] (Table 3).

The Y1 and the Y5 chromosomes are the most frequent of the Y's. Chromosome Y1 showed two significant interactions with sites indicating less Y1 chromosomes than expected in [MIS] and more than expected in [TUC]. Y2 chromosome didn't show any significantly inter-action seeming to stay in equilibrium in all sites. There seems to be more Y3 chromosomes in [BRAZ] and [URUG], than expected.

Chromosome Y5 showed a high interaction effect affecting [BA] while stays according to the expected values in the other sites. Finally, Y6 seems to be a "local" chromosome or population marker in [MIS]: it was not found in any other site. Chromosome Y6 could be found within the best sampled population and its frequency is low.

A similar chromosome to the Y6 variant was described by Selivon[13] for some Brazilian populations of the insect. Solferini & Morgante[7] did not state the number of speci-mens analyzed so they probably associated the absence of heterozygous females with isolation barriers. Beside these authors did not maintain laboratory colonies of Anastrepha species, so it is likely that heterozygous females for different variants were considered to belong to a different sibling species. Even more, we found the Y-chromosome variant of karyotypic form 2 Solferini & Morgante[7] to be an X- chromosome which we named the X2 variant.

A biochemical study on Argentinian and Brazilian populations, demonstrated very low genetic distance between Buenos Aires and Brazil, although the geographic distance is very large.[28]

We could detect 28 sexual karyotypes out of 34 possible different combinations (Figure 2) maintained as laboratory colonies of the species. Our results demonstrate that A. fratercu-lus is single a polymorphic species.

Samplings allowed determination of variant frequencies. Some of them could not be de-tected probably due to sample effect. The fact that the X3 is in low frequency, determines the low probability of X3Y3, X3Y4, X3Y5 combinations. Even though X3 and Y6 were in very low frequency, a combination X3Y6 is detected due to high positive Y6-MIS interaction. Negative interaction is the cause for the low probability of detecting some variants. This is the case for X1Y6, which shows negative interaction with locations.

We studied both Northern and Southern geographic limits of the distribution of *A. fraterculus*: Misiones (Lat 26º56'S) and the south region of Buenos Aires (Lat 34º36'14'' S). The population from Brazil, is located at Lat 31°45' S; Long 52°20'W. Data from ran-dom crossings among those studied samples collected from extreme regions of the insect distribution: [BA] x [BRAZ] and [BA] x [MIS], evidenced that chromosomal variants recom-bine and that they are not reproductive isolation markers. These studies were supported through statistic analysis. We found chromosomal variability among localities but we did not calculate genetic distances. According to Toro[29] classical approach of using genetic distances may present several problems, pointing out that 1) genetic distances ignore within breed variation;2) genetic distances are not suitable for constructing phylogenetic trees and 3) genetic distances vary according to the marker used.

Smith Caldas analyzed a fragment of the mitochondrial gene COI for 15 spe-cies of Anastrepha, 12 of which belong to the fraterculus group. Phylogenetic relationships among the included taxa were inferred using neighbour –joining and maximum parsimony methods. The results of COI indicate the placement of one of the unplaced species *A. acris*, in the fraterculus group. Moreover, the presence of multiple gene pools in the nominal species *A. fraterculus* and the nonmonophyly of *A. fraterculus* are corroborated by data obtained in this study. These authors do not specify the number of specimens used in their study. As stated by Berlocher,[30] intraspecific trees are more informative about population structure when they are mapped onto geographic space.

Alberti el al.,[31] studied the correlation between geographical distribution and genetic variation in natural populations from Argentina and South Brazil sequencing fragments of the mitochondrial gene COII for a total of 28 individuals. Based on Templeton nested method, no clade showed any geographic pattern for the gene COII, indicating lack of sig-nificant association between haplotypic variability and geographic distribution. The analysis of nucleotide substitution distances by Neighbour joining algorithm showed that geographically distant populations exhibit low genetic distances. These authors pointed out that ecological causes of departures from random assignment of chromosomes are to be investigated. We have included some relevant geographical parameters for each location we have worked at and we did not found any relationship among chromosome frequencies and the geographical/climatic measures employed. None of the Spearman's coefficients of correlation calculated resulted statistically significant.[32]

Crossings confirmed a truly recombination of chromosomal variants. The log lineal analysis of data made evident the existence of chromosome x site interaction, coming to light for the existence of sexual karyotypes (combinations) more strongly associated with a particular location. This component of the genetic variance of the studied populations is particularly relevant at present in the face of the constant climatic change. Variants are not genetically isolated but geographically linked to or specialized for a particular situation or locality.[33–35]

Conclusion

1. Chromosomal variants in *A. fraterculus* are associated to different sites.
2. There is variation in the frequencies of each karyotypic variant among sites and there are strong interaction effects between these two factors.
3. Karyotypes showed that variants do not recombine at random.
4. Chromosomal variants in the so called *A. fraterculus* complex do not behave as isolation markers.
5. Our evidence is consistent with the fact that *A. fraterculus* is a single a polymorphic species within the geographical populations studied.

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6. Chromosome x site interaction was quantitatively asserted through ML estimations.

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None

Conflicts of interest

The author declares there are no conflicts of interest.

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