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Monik Oprea

VARIABILIDADE E ESTRUTURA GENÉTICA ESPACIAL EM *GLOSSOPHAGA SORICINA* COM OCORRÊNCIA NO CERRADO

Orientadora: Mariana Pires de Campos Telles
Co-orientadora: Rosane Garcia Collevatti

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Orientadora: Mariana Pires de Campos Telles
Co-orientadora: Rosane Garcia Collevatti

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SUMÁRIO

1. RESUMO…….. 1

2. ABSTRACT.. 3

3. CAPÍTULO 1 - Microsatellite markers in Chiroptera studies: a global analysis of the scientific literature.. 6

Abstract.. 7

Introduction…... 8

Material and Methods... 10

Results……... 11

Discussion…... 18

Literature cited... 20

Supplemental information – Table S1 - Relation of successful cross-amplification …... 27

Supplemental information – List of articles found in the scientometrics study.. 34

4. CAPÍTULO 2 - Isolation and characterization of ten microsatellite loci from Pallas' long-tongued bat *Glossophaga soricina*.. 50

Abstract.. 51

Introduction…... 52

Material and Methods... 52

Results and Discussion... 54

References…... 55

Table 1 - Characterization of the 10 microsatellite loci developed for *Glossophaga soricina* …... 58

5. CAPÍTULO 3 - Local pattern in landscape affects inbreeding and genetic

discontinuity in the Neotropical bat species *Glossophaga soricina*............. 60

Abstract.. 61

Introduction…... 62

Material and Methods.. 64

Results……….. 70

Discussion….. 75

Literature cited.. 78

Supplemental Information – Figure S1 - Posterior probability graphs of the Bayesian clustering simulation……………………………………………. 93

Supplemental Information – Figure S2 - Plots of Q-value for the individuals of 17 populations of *G. soricina* …………………………………………... 94

Supplemental Information – Figure S3a - Correlation between the genetic and geographic distances in 17 populations of *G. soricina*…………… 96

Supplemental Information – Figure S3b - Mantel correlogram ………..... 96

Supplemental information – Table S1 - Location and number of individuals sampled in each population of *G. soricina*……………………………….. 97

Supplemental information – Table S2 - Population subdivision indexes estimates for *G. soricina* based in nine microsatellites markers ……... 98

Supplemental information – Table S3 - Pairwise F_{ST} for 17 subpopulations of *G. soricina* …... 99

Supplemental information – Table S4 - Proportion of membership of each pre-defined population of *G. soricina* in each of the 4 clusters inferred by Bayesian analyses …..100

Supplemental information – Table S5 - Probability of heterozygosity excess for the Wilcoxon test …..105

RESUMO

Marcadores microssatélites são ferramentas importantes para estudos de ecologia molecular, principalmente para estudos sobre morcegos, cujas informações são difíceis de acessar através de observações diretas. No primeiro capítulo, buscamos artigos científicos sobre o uso de microssatélites em morcegos para avaliar o conhecimento atual dos padrões genéticos e revelar os aspectos sociológicos desse conhecimento. Nós observamos que o uso de marcadores microssatélites é relativamente recente e ainda pouco difundido. Muitas questões em ecologia molecular poderiam ser respondidas com um número limitado de marcadores moleculares, como os microssatélites. Isso não só contribuiria para o conhecimento da biologia, mas também para desenhar estratégias efetivas para conservação das espécies de morcegos.

No segundo capítulo, apresentamos o desenvolvimento e caracterização de dez locos de microssatélites para o morcego *Glossophaga soricina*, isolados a partir de uma biblioteca *shotgun*. Foram analisados os genótipos de 67 indivíduos, sendo que o número de alelos por locos variou de 2 a 20, e a heterozigozidade observada e esperada variaram entre 0.015 a 0.606 e entre 0.016 a 0.915, respectivamente. A alta probabilidade de identidade genética (4.369×10^{-8}) e a probabilidade de exclusão de paternidade (0.996) mostraram que os locos de microssatélites desenvolvidos são úteis para estudos de estrutura genética e paternidade em populações naturais de *G. soricina.*

No terceiro capítulo, foram usados nove locos de microssatélites desenvolvidos, juntamente com análises espacialmente explícitas para

acessar a estrutura genética, e verificar como as características da paisagem afetam a diversidade genética de *G. soricina* em 17 localidades do Cerrado brasileiro. Nossos resultados mostraram que populações de *G. soricina* já apresentam altos índices de endogamia em paisagens fragmentadas em pequenas escalas geográficas. Além disso, alguns pares de populações apresentaram descontinuidade genética como resultado da modificação da paisagem.

ABSTRACT

Microsatellite markers are important tools for molecular ecology studies, particularly for bats, whose information is difficult to obtain through direct observations. In the first chapter, we conducted searches for scientific articles about the use of microsatellite markers in bats in order to evaluate the current knowledge about the genetic patterns and also to unravel sociological aspects of this knowledge. We found that the use of microsatellite markers to study bats is quite new and little spread. Many questions in molecular ecology can be addressed with a limited number of polymorphic markers, such as microsatellites. This will not only contribute to the knowledge of the species biology, but also to design effective strategies for conservation of bat species.

In the second chapter, we report the development and characterization of ten microsatellite loci for the bat *Glossophaga soricina* isolated from a shotgun genomic library. Among 67 individuals, the number of alleles per locus ranged from 2 to 20, and the observed and expected heterozygosity ranged from 0.015 to 0.606 and from 0.016 to 0.915, respectively. The high combined probability of genetic identity (4.369×10^{-8}) and probability of paternity exclusion (0.996) showed that the microsatellite loci are useful for population genetic structure and detailed parentage studies in natural populations of *G. soricina.*

In the third chapter, we used the nine developed microsatellite loci and spatially explicit analysis to unravel population genetic structure and how landscape features affected genetic diversity of *G. soricina* at 17 localities in the Brazilian Cerrado. Our results showed that *G. soricina* populations already have higher inbreeding in fragmented landscapes in small geographic scales.

Also, some pairs of populations showed genetic discontinuity as the outcome of landscape modification.

Capítulo 1

Microsatellite markers in Chiroptera studies: a global analysis of the scientific literature

Microsatellite markers in Chiroptera studies: a global analysis of the scientific literature

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Running title: Microsatellite markers in bat studies

Abstract

Microsatellite markers are important tools for molecular ecology studies. These tools are particularly useful for bats, whose information is difficult to obtain through direct observations. We conducted searches for scientific articles about the use of microsatellite markers in bats in Thomson-ISI database in order to evaluate the current knowledge about the genetic patterns and also to unravel sociological aspects of this knowledge. Our search resulted in 106 articles that were published between 1995 and 2011, and we found a total of 56 bats species studied, comprising 10 of the 18 existing families. The main approaches in these studies evaluated aspects related to isolation of microsatellites markers, population genetic structure, gene flow, kinship and behavior. The use of microsatellite markers to study bats is quite new and little spread, with the first work dated from 1995. Since then, the number of articles has grown over the years, although not as expected, considering the number of species recognized for Chiroptera. To understand the social organization and the evolution of bat species, we need to know their reproductive systems, the estimates of relatedness between individuals in social groups, and the patterns of dispersal and gene flow between groups. Therefore, the use of genetic techniques can assist in unraveling such patterns, providing answers to these questions. Many questions in molecular ecology can be addressed with a limited number of polymorphic markers, such as microsatellites. This will not only contribute to the knowledge of the species biology, but also to design effective strategies for conservation of bat species.

Keywords: global trends, molecular ecology, scientometrics, SSR.

Introduction

Molecular markers are important tools for studying the ecology, reproductive biology and behavior of animal populations, particularly for those species whose information is difficult to obtain through direct observations, such as Chiroptera species (Burland and Wilmer 2001). Microsatellite markers have become widely used in molecular ecology because they are co-dominant and multiallelic, displaying high genetic information content (Morgante and Olivieri 1993, Goldstein and Schlotterer 1999). In molecular ecology of mammals, microsatellites have been applied to understand, for example, mating behavior, effective population size and kinship (Selkoen and Toonen 2006).

Bats (Mammalia, Chiroptera) represent the second largest order of mammals (Simmons 2005), comprising species with different life histories, ecologies and morphology (Arita and Fenton 1997). These mammals play important roles in maintaining the diversity of ecosystems, due to their species richness, ecological diversity, large biomass, great mobility and especially, because the interplay in ecological interactions as pollinators, seed dispersers and predators (Kalko *et al.* 1996; Kalko 1998; Boyles *et al.* 2011).

However, bats are quite difficult to study directly because of their nocturnal habits, inaccessible shelters and the difficult to follow the individuals between colonies and breeding sites (Burland *et al.* 2001). Moreover, conservation efforts for most bat species are often impaired by lack of knowledge about their distribution, natural history and genetic diversity (Racey and Entwistle 2003). Therefore, there is a growing need for conservation genetics of this group of mammals (Echenique-Diaz *et al.* 2009), using techniques that are not constrained by direct observation, and in this case genetic markers come into scene as a potential useful tool.

Indeed, in the context of molecular ecology, the use of molecular markers has shed new light on several bat ecology aspects, such as taxonomy and systematic (e.g. Lausen *et al.* 2008; Furman *et al.* 2010); shelters biology, dispersal and distribution patterns of species (Mayer and Helversen 2001; McCracken *et al.* 2006; Furmankiewicz and Altringham, 2007; Bilgin *et al*. 2008; Salgueiro *et al.* 2010); reproduction systems, mating patterns and parenting behavior (Heckel *et al.* 1999; Arnold 2007; Senior *et al.* 2011); phylogeography and phylogeny (O'Brien *et al.* 2007; Rossiter *et al.* 2007; Rued *et al.* 2008); and conservation genetics (Salgueiro *et al.* 2008; Floyd *et al.* 2009). Nevertheless, little is known about the "state of the art" of genetic research on bats, so a scientometric approach may be useful to detect bias and trends in knowledge, as well as to assist in the design of future research. The use of scientometric techniques and tools has often been used to synthesize the progress and identify gaps in scientific research in various areas such as conservation biology (e.g. Bini *et al.* 2005; Fazey *et al.* 2005; Brito and Oprea 2009; Brito 2010), ecology (e.g. Nobis and Wohlgemuth 2004; Carneiro *et al*. 2008) and zoology (e.g. Brito *et al*. 2009; Nabout *et al.* 2010).

In this context, our goal was to assess the state of the art on the use of microsatellite molecular markers in the study of bats, both in respect to knowledge about the genetic patterns in the species and about the sociological aspects of this knowledge. More specifically, we aimed to: (i) determine which species were investigated, and identify the number of microsatellite loci used, (ii) verify the temporal trend in the number of articles on this topic, (iii) identify which countries have the highest number of researchers and articles on this subject, (iv) check if the high income countries have more published articles, (v) locate the biogeographic regions where such approach has been applied to bats, and (vi) verify if threatened species are more studied than non-threatened species.

Material and Methods

The search for scientific papers that used microsatellite markers in bats was conducted in Thomson-ISI database (http://www.isiknowledge.com) because it compiles most of the articles published in refereed journals with peer review process, and is easily accessed by researchers around the world.

The search was performed until the end of 2011 using the routine “Web of Science”, based on the following keywords: (microsatellite* OR SSR OR STR) AND (bat* OR chiroptera). Then we refined our search for the following areas: (Genetics and Heredity OR Biochemistry and Molecular Biology OR Ecology OR Evolutionary Biology OR Zoology OR Biology OR Behavioral Sciences OR Biodiversity Conservation OR Environmental Sciences).

For each article we extracted the following information: (i) the studied species (except species used for testing cross-species amplification); (ii) the aim of study; (iii) for articles on the development of primers, we quantified the number of polymorphic loci; (iv) in cross-species amplification studies, we recorded the species for which they succeeded to amplify the loci; (v) the year of publication; (vii) the affiliation of authors at the time of publication (in case the researchers were affiliated to more than one institution, each institution was recorded independently); (vii) the biogeographic region (*sensu* Pianka 1994) of the study; and (viii) the conservation status of the species following the global red list of threatened species (IUCN 2011).

To analyze the temporal trend in the number of articles per year, we removed the effect of the general trend of increase in publications (Peters 1991), by dividing the number of articles published each year by the total number of articles found in the database from that year.

The relationship between the number of articles and per capita income (U.S. dollars) for the respective countries (27 countries total) was analyzed using Pearson's correlation. Our hypothesis was that countries with higher per capita income have more published articles in Thomson-ISI database (*sensu* Fazey *et al.* 2005). Data on countries per capita income for the year 2011 were obtained from the World Bank database (*The Word Bank Data*, www.worldbank.org/data, accessed on May 4, 2012).

Results

Our search retrieved 106 papers (see Supporting Information) between 1995 and 2011. The papers included a total of 56 bats species, comprising 10 of the 18 existing families (Fig. 1). From the 106 articles, 35 describe microsatellite primers development (Table 1), 67 population genetic structure, one dealt with genomic, and two were on methods.

Methodology used on primers development varied among papers. From the 35 papers, 29 papers used enriched library, 4 used non-enriched genomic library, and the other two merged both approaches at the same time. The number of polymorphic loci ranged from 5 (*Miniopterus schreibersii)* to 22

(*Tylonycteris pachypus*) (Table 1). In addition, 15 papers presented a cross-species amplification test, in a total of 71 species (Supporting Information Table S1).

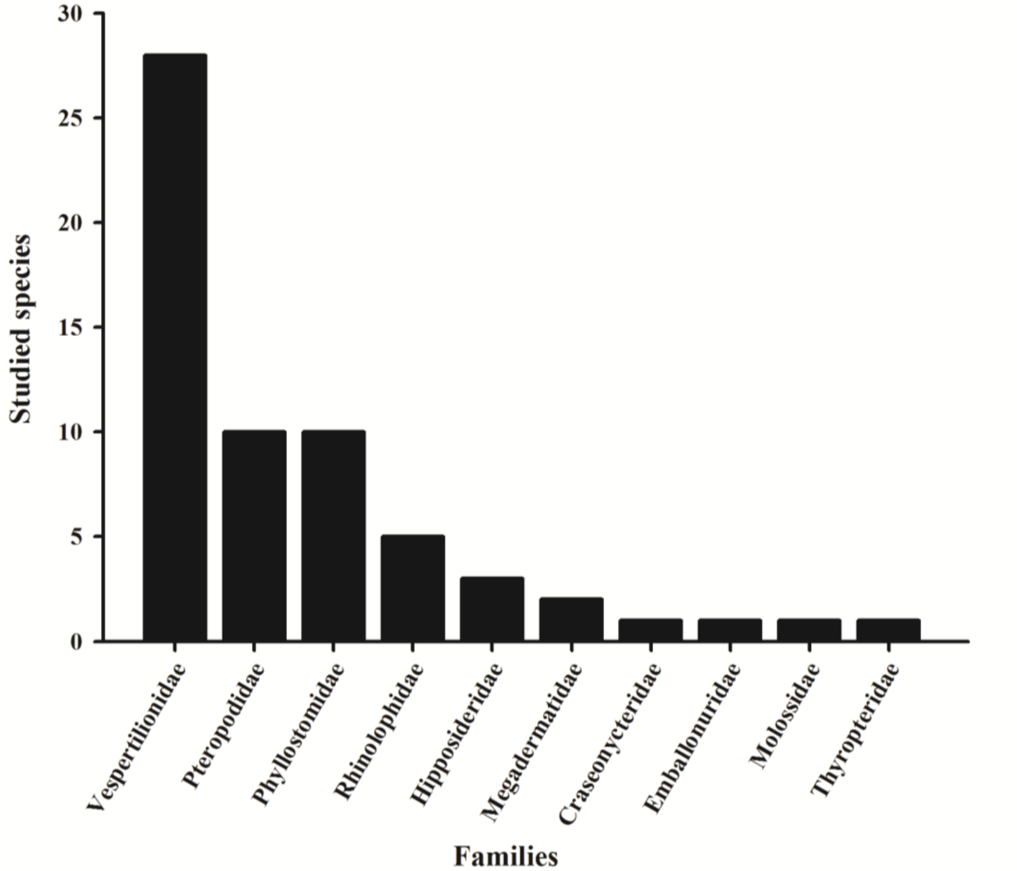

Fig. 1 – Number of studied species in each family, using microsatellites markers.

A significant increase in the number of articles that used microsatellite markers to study bats was observed along the time (16 years) (r=0.61; *p*<0.01) (Fig 2). Most articles were published by researchers from institutions in the United States (35 articles) and United Kingdom (29) (Fig. 3). Countries with largest number of articles are those with the highest income per capita (r=0.45, P<0.05) (Fig. 4).

Table 1 – Bat species studied to develop microsatellites markers, and the number of polymorphic loci, in papers published from 1995 to 2011.

Family / Subfamily / Species	**Polymorphic loci**	**Alleles per locus**
Pteropodidae		
Cynopterus sphinx	9	5-17
Eonycteris spelaea	9	3-22
Rousettus leschenaultia	7	7-16
Rhinolophidae		
Rhinolophus affinis	19	13-15
Rhinolophus ferrumequinum	21	2-18
Rhinolophus hipposideros	14	2-7
Rhinolophus pusillus	13	9-16
Hipposideridae		
Hipposideros armiger	8	3-12
Hipposideros pratti	9	4-14
Hipposideros turpis	6	2-8
Megadermatidae		
Megaderma lyra	10	6-16
Craseonycteridae		
Craseonycteris thonglongyai	16	2-11
Emballonuridae		
Emballonurinae		
Saccopteryx bilineata	12	6-19
Phyllostomidae		
Desmodontinae		
Desmodus rotundus	12	2-13
Phyllonycterinae		
Erophylla sezekorni	12	5-20
Glossophaginae		
Leptonycteris yerbabuenae	12	4-12
Phyllostominae		
Lophostoma silvicolum	7	6-10
Macrotus waterhousii	10	5-20

Carolliinae	10	6-25
Carollia brevicauda		
Stenodermatinae		
Artibeus jamaicensis	14	5-13
Artibeus lituratus	45	N/A
Molossidae		
Molossinae		
Tadarida brasiliensis	9	15-55
Vespertilionidae		
Vespertilioninae	6	12-23
Eptesicus fuscus		
Scotophilus kuhlii	11	5-18
Nyctalus leisleri	11	5-29
Nyctalus noctula	13	7-18
Pipistrellus abramus	10	7-13
Corynorhinus rafinesquii	15	3-13
Plecotus auritus	6	7-24
Chalinolobus tuberculatus	13	5-14
Tylonycteris pachypus	22	2-15
Myotinae		
Myotis myotis	13	2-15
Miniopterinae		
Miniopterus magnater	10	6-27
Miniopterus schreibersii	5	17-20
Kerivoulinae		
Kerivoula papillosa	17	2-19

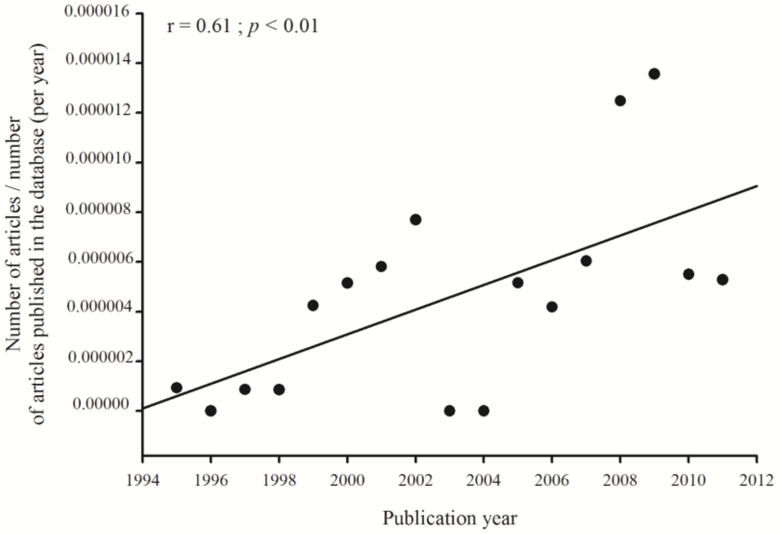

Fig. 2– Number of papers dealing with microsatellites markers in bat studies published by year (1995 to 2011), after removing the increasing trend of articles in the ISI database.

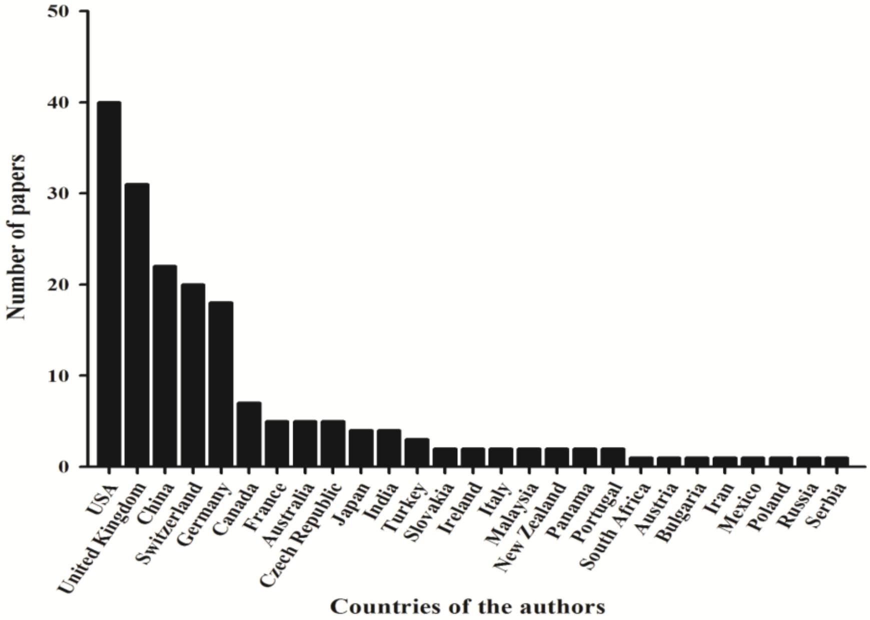

Fig. 3 – Number of papers published from 1995 to 2011 dealing with microsatellites markers in bat studies by authors’ affiliation country.

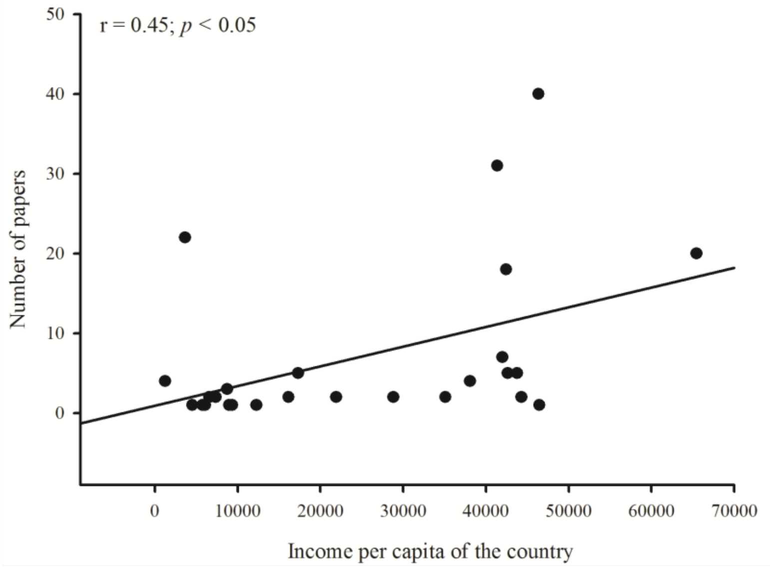

Fig. 4 – Correlation between the number of papers published from 1995 to 2011 dealing with microsatellites markers in bat studies and the income per capita of authors' affiliation country.

The highest number of species studied was from the Palearctic and the Indo-Malayan regions (Fig. 5). However, there was no correlation between the number of species studied with the number of species present in the respective biogeographic region (r^2=-0.09; p = 0.51). From the 106 articles, in 83% at least one of the authors was affiliated to an institution in the same biogeographical region where the bat species occur.

Our search also showed 122 papers dealing with species considered Least Concern and only 2 for Endangered and 1 to Critically Endangered species (Fig. 6). Among the species studied, seven are under some threat category (*Chalinolobus tuberculatus*, *Craseonycteris thonglongyai, Macroderma gigas*, *Myotis capaccinii*, *Nyctalus azoreum*, *Pteropus rodricensis, Rousettus obliviosus*).

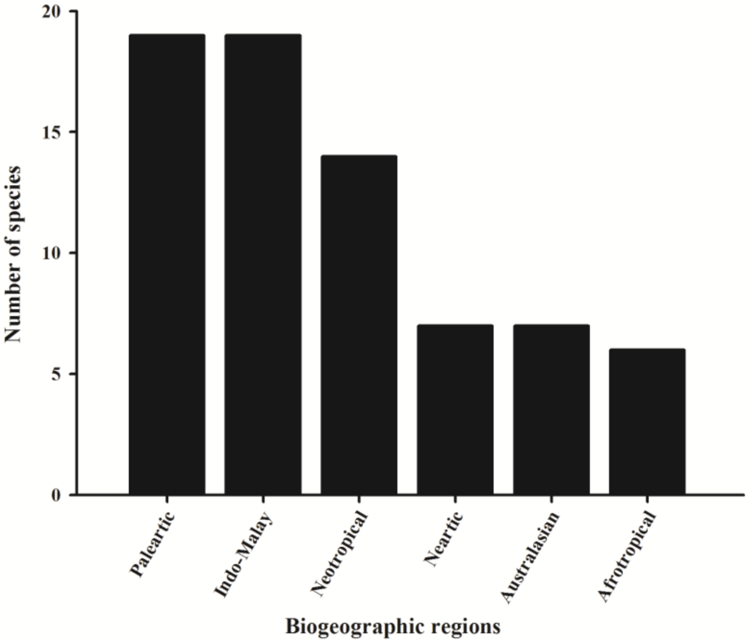

Fig. 5 - Number of studied bat species in each biogeographic region.

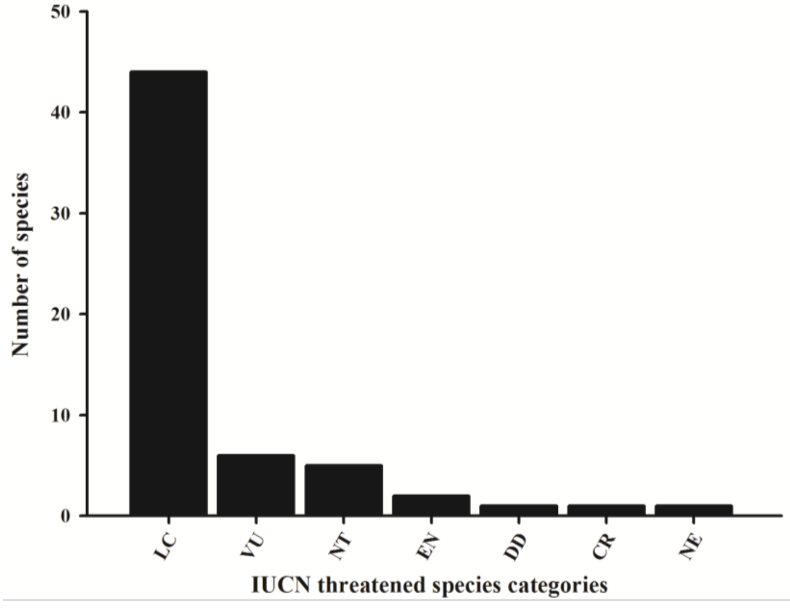

Fig. 6 – Number of studied species in each threatened category proposed by IUCN.

Discussion

Despite being a popular molecular tool, the use of microsatellite is still relatively recent, with the pioneer works dated from 1989. (Tautz 1989; Litt and Luty 1989). Since then, several studies have used this technique in different groups (*e.g.* Baums *et al.* 2005, Bowen *et al.* 2005, Marshall Jr. *et al.* 2009, Quintela-Sabarís *et al.* 2010). Particularly for bats, the use of microsatellite markers is quite new and little spread, with the first work found in the Thomson ISI database dated from 1995. Since then, the number of articles has grown over the years, although not as expected considering the number of species described for Chiroptera (Gonzaga 2013).

Although there are nearly 1200 bat species in the world (Schipper *et al.* 2008), only 56 species were studied using microsatellite markers, and other 71 were used to test cross-species primer amplification. This low number of species studied may be due to the need of microsatellite primer development for each species, when first studied, which is still expensive and demanding (Zane *et al*. 2002).

Regarding the authors affiliation, countries with higher income per capita presented higher number of papers (May 1997; Fazey *et al*. 2005; Nabout *et al.* 2010). These results are consistent with other studies (Fasey *et al*. 2005; Brooks *et al*. 2008; Brito and Oprea 2009; Nabout *et al.* 2010). The presence of developing countries on the list may be explained, in many cases, by the association between researchers in those countries with those from developed countries For example, the presence of Panama is most likely due to the presence of a Smithsonian Institute base in Barro Colorado. The developing countries' absence on the list is quite surprising, as many of developing countries have reported an increase in scientific literature (Fasey *et al.* 2005). This result is somewhat worrisome, since Latin American countries are very rich in bat species (c.a. 350 species). One example is Brazil, which was not responsible for any publication, despite the high number of bat species (174 species). This may be due to the lack of infrastructure, and skilled human resources.

Although the Palearctic (19 species) and the Indo-Malayan (17 species) showed the highest number of studies, they still have very few studies using microsatellites comparing to the number of bat species in the regions (109 and 365, respectively). Another example is the Neotropical region, which comprises more than 300 species and only 11 have some study using microsatellites markers.

Most species used in the studies are classified as “Least Concern” by the world list of threatened species (IUCN 2011). This is most likely due to the sampling facility because Least Concern species are usually widely distributed and abundant, since population genetic studies require large scale samplings and high number of individuals per locality. Nevertheless, from the 687 Least Concern species, only 43 had some study with microsatellites. We believe that the studies on molecular ecology based on microsatellite markers for the 172 species listed as threatened may contribute to their management and conservation. Moreover, generating such information for the species listed as Data Deficient may improve the knowledge about them and contributes to their proper classification into one of the threat categories.

In conclusion, we believe that one possible reason for the still limited number of articles on this subject is the lack of trained researchers and infrastructure in most underdeveloped countries. Incentives for young researchers to train such skills in institutions that master the technique and have skilled personnel should be implemented by developing countries (e.g. Ciência sem fronteiras programme in Brazil). Besides that, researchers should devise a strategic plan to move forward the use of microsatellite in bats, identifying priority species, places and questions. For example, the technique is still underexplored for conservation issues, as suggested by the small number of threatened species appearing in our results.

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Supplemental Information

Table S1 – Relation of successful cross-amplification. In this relation it was not considered if there was polymorphism.

Primer development	Cross-amplification	Number of amplified primers	Reference
Rhynchonycteris naso	*Saccopteryx bilineata*	4	Nagy et al. (2010)
	Saccopteryx leptura	4	
	Balantiopteryx plicata	2	
	Cormura brevirostris	2	
Leptonycteris yerbabuenae	*Leptonycteris curasoae*	12	Ramirez et al. (2011)
	Leptonycteris nivalis	9	
	Choeronycteris mexicana	8	
Miniopterus schreibersii	*Miniopterus aelleni*	8	Wood et al. (2011)
	Miniopterus australis	20	
	Miniopterus brachytragos	7	
	Miniopterus fraterculus	7	
	Miniopterus fuliginosus	18	
	Miniopterus gleni	7	
	Miniopterus griffithsi	15	
	Miniopterus griveaudi	8	

	Miniopterus inflatus	16	
	Miniopterus macrocneme	17	
	Miniopterus magnater	19	
	Miniopterus mahafaliensis	17	
	Miniopterus majori	7	
	Miniopterus medius	20	
	Miniopterus minor	7	
	Miniopterus natalensis	7	
	Miniopterus petersoni	12	
	Miniopterus propitristis insularis	4	
	Miniopterus tristis tristis	16	
Artibeus lituratus	*Artibeus planirostris*	52	McCulloch and Stevens (2011)
	Artibeus fimbriatus	54	
	Artibeus phaeotis	45	
	Enchisthenes hartii	50	
	Sturnira lilium	47	
	Carollia perspicillata	45	
Rhinolophus ferrumequinum	*Rhinolophus monoceros*	8	Dawson et al. (2004)
	Rhinolophus celebensis	7	
	Rhinolophus lepidus	7	
	Rhinolophus affinis	7	

	Rhinolophus stheno	6	
	Rhinolophus trifoliatus	6	
	Rhinolophus euryotis	5	
	Hipposideros diadema	5	
	Hipposideros larvatus	3	
	Hipposideros cervinus	1	
	Hipposideros ridleyi	1	
	Hipposideros bicolor	1	
	Hipposideros dinops	1	
	Myotis nattereri	1	
Plecotus auritus	*Plecotus austriacus*	6	Burland et al. (1998)
	Corynorhinus townsendii	5	
	Pipistrellus pipistrellus	3	
	Pipistrellus kuhli	3	
	Pipistrellus hesperus	1	
	Pipistrellus subflavus	2	
	Pipistrellus mimus	2	
	Pipistrellus stenopterus	2	
	Myotis daubentonii	3	
	Myotis nattereri	4	
	Nyctalus noctula	1	

Carollia brevicauda	*Carollia castanea*	8	Bardeleben et al. (2007)
	Carollia perspicillata	7	
Rhinolophus pusillus	*Rhinolophus macrotis*	10	Hua et al. (2009)
	Rhinolophus affinis	8	
	Rhinolophus pearsonii	10	
	Rhinolophus ferrumequinum	8	
	Rhinolophus. sinicus	7	
Hipposideros armiger	*Hipposideros larvatus*	8	Guo et al. (2008)
	Hipposideros pomona	7	
	Hipposideros pratti	7	
	Rhinolophus pusillus	4	
	Rhinolophus pearsonii	3	
	Rhinolophus macrotis	2	
	Rhinolophus affinis	2	
Chalinolobus tuberculatus	*Chalinolobus gouldii*	9	Dekrout et al. (2009)
Tylonycteris pachypus	*Tylonycteris robustula*	7	Yin et al. (2009)
Eptesicus fuscus	*Antrozous pallidus*	8	Vonhof et al. (2002)
	Corynorhinus townsendii	8	
	Lasionycteris noctivagans	5	
	Lasiurus borealis	3	
	Lasiurus cinereus	4	

	Myotis californicus	4	
	Myotis ciliolabrum	3	
	Myotis evotis	3	
	Myotis lucifugus	4	
	Myotis volans	3	
	Pipistrellus subflavus	6	
Thyroptera tricolor	*Thyroptera lavali*	11	Vonhof et al. (2001)
	Natalus stramineus	1	
	Natalus micropus	2	
	Natalus tumidirostris	1	
	Furipterus horrens	3	
	Eptesicus fuscus	1	
	Lasionycteris noctivagans	2	
Artibeus jamaicensis	*Saccopteryx bilineata*	3	Ortega et al. (2002)
	Nycteris thebaica	4	
	Rhinolopus darlingi	4	
	Mormoops megalophylla	4	
	Pteronotus parnellii	5	
	Phyllostomus hastatus	9	
	Glossophaga soricina	8	
	Leptonycteris nivalis	9	

	Carollia perspicilliata	9	
	Uroderma bilobatum	13	
	Lasiurus borealis	2	
	Myotis adversus	2	
	Nycticeinops schlieffeni	2	
	Chaerephon pumilus	2	
Miniopterus schreibersii	*Miniopterus fraterculus*	5	Miller-Butterworth et al. (2002)
	Chaerephon pumila	2	
	Chaerephon ansorgei	2	
Nyctalus noctula	*Nyctalus leisleri*	8	Mayer et al. (2000)
	Pipistrellus pipistrellus	7	
	Pipistrellus mediterraneus	7	
	Chaerephon kuhli	7	
	Chaerephon nathusii	6	
	Eptesicus nilssoni	7	
	Vespertilio murinus	7	
	Plecotus austriacus	7	
	Plecotus auritus	6	
	Myotis myotis	3	
	Myotis bechsteini	2	
Rhinolophus sinicus	*Rhinolophus ferrumequinum*	5	Liu et al. (2009)

	Rhinolophus macrotis	8	
	Rhinolophus affinis	8	
	Rhinolophus pearsoni	8	
	Rhinolophus pusillus	8	
Hipposideros pratti	*Rhinolophus affinis*	3	Liu et al. (2008)
	Rhinolophus macrotis	3	
	Rhinolophus pearsonii	2	
	Rhinolophus pusillus	2	
	Hipposideros larvatus	8	
	Hipposideros pomona	5	
	Hipposideros armiger	8	
Saccopteryx bilineata	*Balantiopteryx plicata*	3	Heckel et al. (2000)
	Centronycteris maximilliani	3	
	Rhynchonycteris naso	7	
	Saccopteryx leptura	11	

Supplemental Information

List of articles found in the scientometric analysis

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Capítulo 2

Isolation and characterization of ten microsatellite loci from Pallas' long-tongued bat *Glossophaga soricina*

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Isolation and characterization of ten microsatellite loci from Pallas' long-tongued bat *Glossophaga soricina*

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Running title: Ten microsatellite loci from *Glossophaga soricina*

Abstract

Glossophaga soricina is a widespread Neotropical nectarivorous bat. Herein we report the development and characterization of ten microsatellite loci isolated from a shotgun genomic library. Among 67 individuals, the number of alleles per locus ranged from 2 to 20, and the observed and expected heterozygosity ranged from 0.015 to 0.606 and from 0.016 to 0.915, respectively. The high combined probability of genetic identity (4.369×10^{-8}) and probability of paternity exclusion (0.996) showed that the microsatellite loci are useful for population genetic structure and detailed parentage studies in natural populations of *G. soricina.*

Keywords: Cerrado, Chiroptera, conservation, genetic diversity, Neotropical bat, shotgun genomic library.

Introduction

Glossophaga soricina (Chiroptera: Phyllostomidae) is one of the most common and widespread bats in the Neotropics (Simmons 2005). It feeds mainly on floral nectar, being an important pollinator of many Neotropical plants (Nogueira et al. 2007). Despite the current knowledge about species life history there is still much to know about population genetic structure, and also kinship and mating system within social groups. Microsatellite markers may provide useful tools for genetic studies on animals' populations, especially to those where information is difficult to achieve by direct observations, like bats (Bryja et al. 2009). Hence, we report the development and characterization of a set of microsatellite loci for *G. soricina,* as a tool to study population genetic structure, patterns of gene flow and mating system of this species.

Materials and methods

Microsatellites were developed from a shotgun genomic library. For this, DNA from blood of one individual of *G. soricina* was extracted following the protocol described by Sambrook and Russell (2001). The DNAwas sheared using a sonicator (120W for 1h45min) to obtain fragments of 500bp to 1kb. Fragments were recovered, cloned into pMOSBlue vector using the Blunt-ended PCR Cloning Kit® (GE HealthCare, Sweden), and transformed into chemically pMOSBlue® competent cells (GE HealthCare). Recombinant clones were selected in Luria-Bertani (LB) plates, containing ampicillin and X-Gal. For plasmid DNA extraction, recombinant clones were grown overnight in liquid ampicillin LB media, following the protocol described in Sambrook and

Russell (2001). Finally, the DNA inserts were sequenced on a 3100 automated DNA sequencer (Applied Biosystems, CA), using the U19 primer and the DYEnamicET terminator kit (GE Healthcare), according to the manufacturer´s instruction. Sequences were analyzed for their nucleotide content and filtered by their quality and length (phred value ≥20; length ≥150). The selected reads were screened for microsatellites using the WEBSAT software (Martins et al. 2009) and primer were designed using the software Primer3 (Rozen and Skaletsky 2000).

Wing membranes from 67 individuals of *G. soricina* were sampled for loci characterization. DNA was extracted using the DNeasy Tissue Kit (QIAGEN). Microsatellite amplifications were performed in a 15µl final volume, containing 12.5ng template DNA, 0.13µM of each primer, 1U Taq DNA polymerase (Phoneutria, BR), 0.21 mM of each dNTP, 0.21 mg BSA and 1X reaction buffer (10mM Tris-HCl, pH 8.3, 50mM KCl, 1.5mM $MgCl_2$) with the following conditions: 94^0C for 5 min (1 cycle), followed by 30 cycles of 94^0C for 1 min; annealing temperature (see Table 1) for 1 min; 72^0C for 1 min; and 72^0C for 7 min (1 cycle). To detect the polymorphisms, we used 6% denaturing polyacrylamide gels stained with silver nitrate (Creste et al. 2001) and the alleles were sized by comparison to a 10-bp DNA ladder standard (Invitrogen, USA).

The number of alleles per locus, the observed and expected heterozygosity under Hardy–Weinberg equilibrium (HWE) (Nei 1978), and inbreeding coefficient (Weir and Cockerham 1984) were estimated using FSTAT 2.9.3.2 (Goudet 2002). The probability of genetic identity (Paetkau et al. 1995) and paternity exclusion probability (Weir 1996), for each polymorphic locus and overall loci, were estimated using Identity 1.0 (Wagner and Sefc 1999).

Results and Discussion

We sequenced 2,112 clones and 474 presented microsatellite sequences. Primers were designed for 15 sequences and 10 amplified successfully (Table 1). All loci were polymorphic with numbers of alleles ranging from 2 to 20 (Table 1). Observed heterozygosities ranged from 0.015 to 0.606, whereas expected heterozygosities ranged from 0.016 to 0.915 (Table 1). Other studies using non-enriched libraries also observed low heterozigozity for bat species (e.g. Rossiter et al. 1999; Mayer et al. 2000). The deviation from Hardy–Weinberg equilibrium observed in some loci (Table 1) and linkage disequilibrium between loci GS04 and GS13 are most likely due to Wahlund effect, because of the sampling of individuals from different social groups. However, the high combined paternity exclusion and low genetic identity probabilities overall loci (Table 1) demonstrate that the developed microsatellite loci are suitable for population genetic structure and detailed parentage studies in natural populations.

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Table 1 - Characterization of the 10 microsatellite loci developed for *Glossophaga soricina*, based on 67 individuals.

Locus	Repeat motif	Primer sequence (5’-3’)	T_a	A	H_o	H_e	f	I	Q
GS02	(AG)8	(F) TGGACCAACATGACAATATG (R) CACATATAGGAATCAACCAATG	56	6	0.224	0.537	0.582	0.266	0.303
GS03	(TG)20	(F) GGATCTGGGTCCAAAATAAA (R) ACAGACCCAAGTACCCTACAG	56	20	0.666	0.915	0.272	0,016	0,810
GS04	(TG)7	(F) CCGACTGAGGTCAAGTTGTA (R) CTGATGCAGGAGTATGGAAA	56	6	0.358	0.735	0.512	0.117	0.493
GS05	(TC)24	(F) TGCTTTACTTCTCCCAACAA (R) CCAATCACATGGTGAGAGC	56	5	0.606	0.663	0.086^{NS}	0.161	0.423
GS08	AC(8)	(F) TAGACCCCATAAACCTGGAC (R) AGAAAATTAGCTCAGAGGAACC	66	2	0.016	0.016	0.000^{NS}	0.967	0.008
GS10	CAAA(6)	(F) AATTCGAGCTCCCCTACC (R) TCCTCCCAACAGTTTTATGA	56	3	0.015	0.515	0.970	0.363	0.197
GS12	AC(19)	(F) AACGGGAAGGACATATCAAT (R) AGGCATTGAGCAGTTTCTAGT	60	13	0.415	0.897	0.537	0.023	0.771
GS13	TG(7)	(F) CCGACAGAGGTCAAGTTGTA (R) CTGATGCAGGAGTATGGAAA	64	4	0.363	0.309	-0.176^{NS}	0.511	0.148
GS14	AGGA(5)	(F) ATAAATGGTGGTGGAAGGAC (R) GACTGGGAGACATGGGTAAC	64	3	0.338	0.316	-0.072^{NS}	0.515	0.137
GS15	AG(8)	(F) GCACACCAAAGTGACAGACT (R) CACATATAGGAATCAACCAA	60	4	0.184	0.586	0.685	0.243	0.316
All							0.419	4.369798 e-008	0.996

T_a = annealing temperature; A = number of alleles; H_o = observed heterozygosity; H_e = expected heterozygosity; f = fixation index; I = probability of genetic identity; Q = paternity exclusion probability. Value followed by NS is not significant ($P > 0.005$).

Capítulo 3

Local landscape pattern affects inbreeding and genetic discontinuity in the Neotropical bat species *Glossophaga soricina*

Local landscape pattern affects inbreeding and genetic discontinuity in the Neotropical bat species *Glossophaga soricina*

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Abstract

Bat species may present low levels of genetic differentiation among populations due to historical long distance dispersal precluding the detection of anthropogenic disturbance and habitat fragmentation effects on genetic diversity. However, animal mobility and dispersal may be affected by habitat loss and fragmentation. We analyzed 216 individuals of *Glossophaga soricina* at 17 localities in the Brazilian Cerrado, using nine microsatellite loci and spatially explicit analyses to unravel population genetic structure and how landscape features affected genetic diversity. Population differentiation was low, but significant, suggesting long distance gene flow at this geographical scale. Despite the high anthropogenic disturbance and fragmentation in central Brazil, *G. soricina* still presents high genetic diversity. However, our results showed that *G. soricina* populations already have higher inbreeding in fragmented landscapes in small geographic scales. Also, some pairs of populations showed genetic discontinuity as the outcome of landscape modification. These findings are important for *G. soricina* conservation planning that should consider the local pattern of landscape fragmentation to recover natural vegetation, improving the connectivity among populations and potentially increasing dispersal.

Keywords: Chiroptera, habitat fragmentation, microsatellites

Introduction

Species with high mobility may present low levels of genetic differentiation among populations due to historical long distance dispersal (e.g. Wilkinson & Fleming 1996, Russel et al. 2005, Côté et al. 2012) precluding the detection of anthropogenic disturbance and habitat fragmentation effects on genetic diversity. However, animal mobility and dispersal may be affected by habitat loss and fragmentation (e.g. Awade & Metzger 2008, Puttker et al. 2011). Landscape features, such as matrix quality and distance among habitats may isolate populations, limiting the dispersal among populations (Umetsu et al. 2008; Boscolo & Metzger 2011), and thus population connectivity (e.g. Coulon et al. 2004, Johansson et al. 2007). Population isolation may lead to a decrease in the number of alleles due to genetic drift and limited gene flow may result in small effective population size and inbreeding (Gilpin and Soule, 1986; Frankham, 2003). The consequent loss in genetic variability may reduce reproductive success and survival, increasing the risk of extinction (Lande, 1988; Lacy 1997, Reed and Frankham, 2003).

Although bats may be less vulnerable to the deleterious effects of fragmentation than less vagile species (e.g. Campbell et al. 2009), recent studies have demonstrated different responses to this process (e.g. Cosson et al. 1999, Rossiter et al. 2000, Gorresen and Willig 2004, Kerth and Petit 2005, Bernard and Fenton 2007, Estrada-Villegas et al. 2010). Ecological interactions are also involved in the way fragmentation affects bat species, since ecological processes such as pollination and seed dispersal will also be affected, compromising ecosystem dynamics and regeneration (Bernard and

Fenton 2007).

Due to their high vagility, bats may show high levels of gene flow and low genetic differentiation among populations (Burland and Wilmer 2001). However, besides physical barriers to dispersal, reproductive behavior and historical patterns of colonization may also be responsible for restricted gene flow (Burland and Wilmer 2001). Several studies have been showing significant population substructure in bat species (e.g. Arnold 2007, Furmankiewicz and Altringham 2007, Salgueiro et al. 2008, Biollaz et al. 2010, Ripperger et al. 2012).

Glossophaga soricina (Glossophaginae) is one of the most common and widespread nectarivorous bats in Brazil (Marinho-Filho and Sazima 1998), and it is responsible for the pollination of many plant species (Sazima et al. 1999, Tschapka et al. 1999, Muchhala 2006). The species can also feed on insects and fruits (Willig et al. 1993, Bredt et al. 1999), depending on the availability of resources (Tschapka 2004, Lopez and Vaughan 2007). This bat uses a large variety of habitats (from primary forests to urban environments), because of the versatility in the use of roosts (caves, trees, human-made structures) and diet (Nogueira et al 2007). They usually form colonies with males and females, but sometimes, maternity colonies with females and pups are also found (Webster 1983, Willig 1983). Adult males may defend harens and juveniles from both sexes disperse from their natal colony (Pink 1996).

Since *G. soricina* is a common and widespread species, one might expect lack of habitat fragmentation effects on genetic diversity. However, there are evidences of deleterious effects of habitat fragmentation on genetic variability of common and widespread species (Wayne et al 1992, Johansson et al. 2007, Delaney et al. 2010). In addition, more than half of the Cerrado biome has been transformed into pastures and crops (Klink and Machado 2005, Silva et al. 2006) jeopardizing species long-term conservation.

In this study, we used nine microsatellite loci and spatially explicit analyses to understand population genetic structure in *G. soricina* in the Brazilian Cerrado biome, and also how landscape features affected population genetic diversity and differentiation.

Material and Methods

Populations and tissues sampling

Glossophaga soricina was sampled at 17 localities (216 individuals) in the Brazilian Cerrado (Figure 1, Supplemental Information Table S1). We used 3mm diameter tissue samples (wing membrane, uropatagium or liver) preserved in 95% alcohol at 4^{o}C.

Microsatellite genotyping

DNA was extracted following the protocol of Miller et al. (1988). Individuals were genotyped at nine microsatellite loci (Supporting Information Table S2) previously developed for *G. soricina* (Oprea et al. 2012). Forward primers were labeled with fluorescent dyes (6-FAM, HEX and NED, Applied Biosystems, CA) and amplifications were conducted in 15 µl reaction volume, containing 0.13 µM of each primer, 1U Taq DNA polymerase (Phoneutria, BR), 0.21 mM of each dNTP, 1X reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCL_2$), 0.21 mg of BSA and 12.5 ng of template DNA.

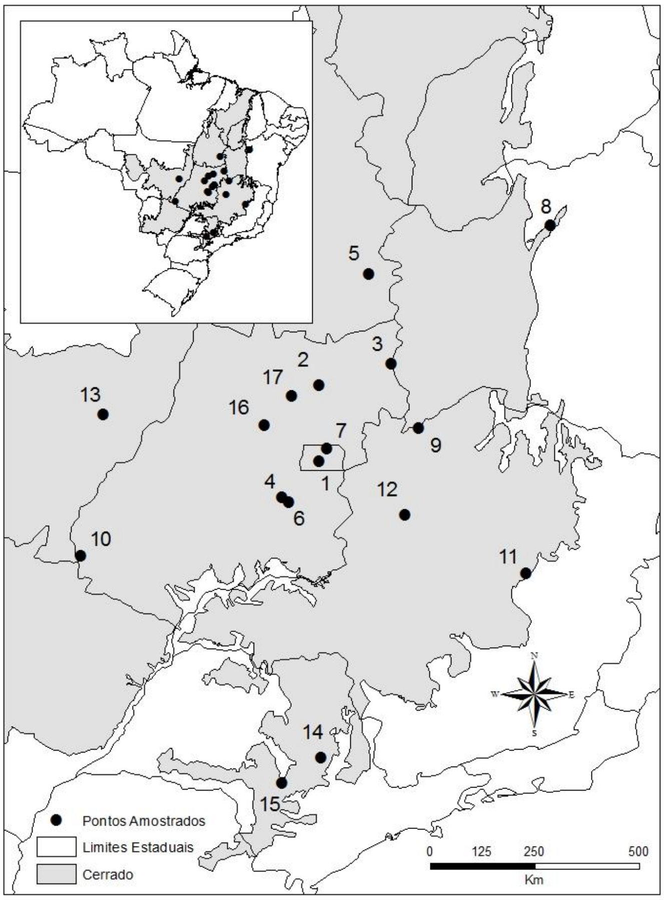

Figure 1. Sampling localities of the 17 populations of *Glossophaga soricina* in Brazilian Cerrado Biome. 1 – Brasília - DF; 2 – Parque Nacional da Chapada dos Veadeiros – GO; 3 – Parque Estadual de Terra Ronca – GO; 4 – Silvânia – GO; 5 – Dianápolis – TO; 6 – Vianópolis – GO; 7 – Estação Ecológica de Águas Emendadas – DF; 8 - Parque Nacional da Chapada Diamantina – BA; 9 – Parque Nacional Grande Sertão Veredas – MG; 10 – Parque Nacional de Emas – GO; 11 – Diamantina – MG; 12 - Brasilândia de Minas – MG; 13 – Nova Xavantina – MT; 14 – Estação Ecológica de Itirapina – SP; 15 – Pratânia – SP; 16 – Barro Alto – GO; 17 – Niquelândia – GO.

Amplifications were performed using Verity® thermal cycler (Applied Biosystems, CA) with an initial denaturation of 94 ^{o}C for 5 min, followed by 45 cycles of 1 min at 94 ^{o}C, 1 min at the annealing temperature (according to each primer) and 1 min at 72 ^{o}C, with a final extension step of 30 min at 72 ^{o}C to control for Taq polymerase adenylation. The PCR products were subjected to electrophoresis on an ABI Prism 3100 automated DNA sequencer (Applied Biosystems, CA) and were sized by comparison to GeneScan ROX 500 size standard (Applied Biosystems, CA) using GeneMaper® v4.1 software (Applied Biosystems, CA).

Electropherograms were visually checked to minimize genotyping error due to stutter band and drop out and genotypes were analyzed in Micro-Checker 2.2.3 software (Van Oosterhout et al. 2004) to check for null alleles and other errors.

Genetic diversity and population differentiation

Populations were characterized for genetic diversity, estimated by expected heterozygosities under Hardy-Weinberg equilibrium (H_e, Nei 1978), and for polymorphism, based on the mean number of alleles per locus (A) and allelic richness (R_s, Mousadik and Petit, 1996). Observed heterozygosities and inbreeding coefficients (f) were also estimated for each population to test for deviation from Hardy-Weinberg equilibrium. All analyses and randomization based tests were performed with the software FSTAT 2.9.3.2 (Goudet et al. 1996; Goudet 2002).

To verify if populations are genetically structured we estimated Wright's F-statistics (Wright, 1951), F_{IS}, F_{IT} and F_{ST} , from an analysis of variance of allele frequencies (Weir & Cockerham 1984) implemented in the software FSTAT 2.9.3.2 (Goudet 2002). We also estimated R_{ST} statistics (Slatkin 1995), analogous to F_{ST}, based on the stepwise mutation model, and tested the hypothesis that $F_{ST} = R_{ST}$ using the software Spagedi 1.3 (Hardy & Vekemans 2002).

We also used a Bayesian clustering method implemented in the software Structure 2.3.4 (Pritchard et al. 2000), to identify genetically homogeneous groups within our samples. We estimated *a posteriori* probabilities of K (number of groups) ranging from 1 to 17 (corresponding to each sampled locality), assuming uniform prior values, admixture model of ancestry and correlated allele frequencies. The analyses were based on 5 independent runs with 100,000 iterations following a burn-in-period of 50,000 iterations. We used Structure Harvester (Earl and von Holdt 2012), which implements the Evanno method (Evanno et al. 2005) to detect the number of K that best fit the data.

We grouped the individuals in the 4 populations assigned by Structure, and run a Wilcoxon sign-rank test implemented in the software Bottleneck version 1.2.02 (Cornuet and Luikart, 1997) to detect recent signal of bottleneck could be detected. The Wilcoxon test was chosen because it is robust with fewer than 20 loci (Piry et al. 1999). Populations that experienced a bottleneck may show an excess of heterozygosity compared to the expected heterozygosity under mutation-drift equilibrium (Maruyama and Fuerst, 1985). Bottleneck analysis was run under the Infinite Allele Mutation Model (IAM), the Stepwise Mutation Model (SMM) and the Two-phase Mutation Model (TPM) with 30% TPM and 70% stepwise mutation model to verify the sensitivity of the analysis to the mutation model.

Spatial patterns and landscape analyses

To test for correlation between geographic distance (natural logarithm) and genetic differentiation (linearized pairwise F_{ST}, Slatkin 1995) we performed a Mantel test using ADE4 package (Dray & Dufour 2007) in R software. A Mantel correlogram was also obtained to better understand the relationship and sampling scale effects, using the software SAM v.4 (Rangel et al. 2010). Statistical significance of matrix correlations was established using 10,000 random permutations for both analyses.

Pairwise-F_{ST} was used to assess discontinuities in multivariate genetic data across the geographic space (Manel et al. 2003) using a Delaunay network (Legendre and Legendre 1998) implemented in the software SAM v.4 (Rangel et al. 2010). The 17 subpopulations were linked and we evaluated discontinuity mapping 10% of the highest ratios between genetic and geographic distances along the Delaunay network (Legendre and Legendre 1998; Manel et al. 2003).

To analyze landscape features effects on population genetic variability and differentiation, we drew landscape-buffers of 2.0 and 5.0 km-radius around each sampled location using the software ArcMap v.9.3. We chose these landscape-buffer sizes due to the *G. soricina* home range (Fleming et al. 1972, Heithaus et al. 1975, Aguiar 2006) and also to verify differences in the analysis scale.

Each landscape-buffer was analyzed to characterize and classify matrix type (natural vegetation remnants, urban area, agricultural area or pastureland, see Supporting Information, Table S1). We also calculated the percentage of each matrix type inside each buffer with V-LATE tool implemented in ArcMap v.9.3. Relationships among the genetic parameters and the matrix type or the percentage of the natural vegetation remnants were analyzed using the BBMLE (Bolker 2012) and GAM (Hastie 2013) packages implemented in R software. We tested three different models to find which fits best to each variable: Generalized Linear Model (glm), Generalized Addition Models (gam), and Maximum Likelihood Estimation (mle2). We used Akaike Information Criterion (AIC) approach to choose the best fitted model. The model with lower AICc (AIC corrected by sample size and number of parameters, Burnhan & Anderson 2002) was considered more plausible to explain observed patterns. The difference between AICc for the model *i* and the smallest observed AICc (ΔAICc) was calculated for each model. Models with ΔAICc<2 are equally plausible to explain the observed pattern (Zuur et al. 2009). The Akaike's weight of evidence (wAICc) was also obtained to verify the relative contribution of each model (Burnhan & Anderson 2002).

We also analyzed the relationship between pairwise-F_{ST} and landscape features using a multiple Mantel test (MRM) implemented in ECODIST package (Goslee and Urban 2012) in R software. In this case, we connected all possible population pairs and drew a 5 km buffer to classify matrix type and percentage of natural vegetation remnants, and calculate the matrix resistance. Matrix resistance between each pair of location was classified based on the information available in the literature (e.g. Estrada et al. 1993). We decided to be conservative to assign resistance values, since bats present great vagility. Resistance values were: vegetation and water bodies (resistance = 1), agriculture and urban areas (resistance = 2), pastures (resistance = 3).

Results

Genetic diversity and population differentiation

Mean number of alleles per locus ranged from 3.6 to 5.6 (Table 1). Populations presented very similar levels of genetic diversity and polymorphism (Table 1), despite the differences in sample size. The observed heterozygosity was lower than the expected under Hardy-Weinberg equilibrium for most populations, with inbreeding coefficient significantly different from zero (Table 1).

Table 1 - Genetic variability for the 17 populations of *Glossophaga soricina* in Brazilian Cerrado Biome, based on nine microsatellite loci.

Population	N	A	R_S	H_E	H_O	f
1	10	3.667	2.860	0.563	0.578	-0.026
2	22	5.667	2.833	0.465	0.427	0.081*
3	10	4.667	3.281	0.601	0.482	0.198**
4	14	4.333	3.046	0.549	0.465	0.153*
5	15	4.556	3.074	0.590	0.376	0.347**
6	10	3.778	2.781	0.562	0.272	0.516**
7	7	3.778	3.033	0.535	0.524	0.020
8	10	4.222	2.916	0.492	0.463	0.058
9	13	4.444	2.804	0.495	0.480	0.031
10	18	4.889	3.130	0.578	0.549	0.051
11	14	4.889	3.190	0.585	0.514	0.122*
12	9	3.667	2.848	0.559	0.391	0.301**
13	10	4.111	3.043	0.584	0.500	0.144*
14	9	4.000	2.955	0.529	0.481	0.089
15	8	4,333	3.133	0.576	0.472	0.180*
16	22	5.667	3.354	0.600	0.492	0.180**
17	15	4.667	3.094	0.559	0.572	-0.024

N = number of individuals analyzed; A = mean number of alleles; R_S= allelic richness based on minimum sample size (4 individuals); H_E = expected heterozygosity; H_O = observed heterozygosity; f= inbreeding coefficient (*P < 0.05, **P < 0.01);

Population differentiation was low, but significant (F_{ST} =0.022, SE = 0.005, P < 0.0001), suggesting long distance gene flow at this geographical scale. We also found high amounts of inbreeding within populations (F_{IS} = 0.138, SE = 0.092, P < 0.0001) and non-random mating among populations (F_{IT} = 0.157, SE = 0.092, P < 0.0001) (see Supplemental Information Table S2). R_{ST} overall population and loci was also low (R_{ST} = 0.0905, SD = 0.011, P < 0.0001). We found a significant difference between F_{ST} and R_{ST} (P = 0.0007).

Bayesian clustering of individuals showed the highest probabilities (L(D|K)) and lowest confidence intervals when populations were grouped into four clusters (K = 4) (Supplemental Information Figure S1, Table S4). We also found higher ΔK for K = 13. However, because the individuals in each inferred cluster for K = 13 were evenly distributed (Q-value similar among clusters), we considered K = 4 groups (Supplemental Information Figure S2), following Pritchard et al. (2000)**.**

The analysis of population reduction using Bottleneck was performed considering the four groups obtained with Bayesian clustering. No evidences of genetic bottleneck were detected for the three mutation models (see Supplemental Information Table S5).

Spatial patterns and landscape analyses

Genetic differentiation is not correlated with geographical distance (Mantel test, P = 0.844; Supplemental Information Figure S3a). The lack of relationship is not due to spatial scale because correlation was not significant in any distance classes (see Supplemental Information Figure S3b).

We found evidences of discontinuity between four pair of populations: 1–6, 1–7, 2–17, and 4–6 (Figure 3). The areas of discontinuity correspond, in the land use map, to a large urban area surrounding population 1, which could explain the genetic discontinuity between this locality and subpopulations 6 and 7, and a large area of pasture and agriculture between populations 1 and 6. The area between populations 4 and 6 is an agricultural area, and locality 17 is in a highly disturbed area with intense mining activity.

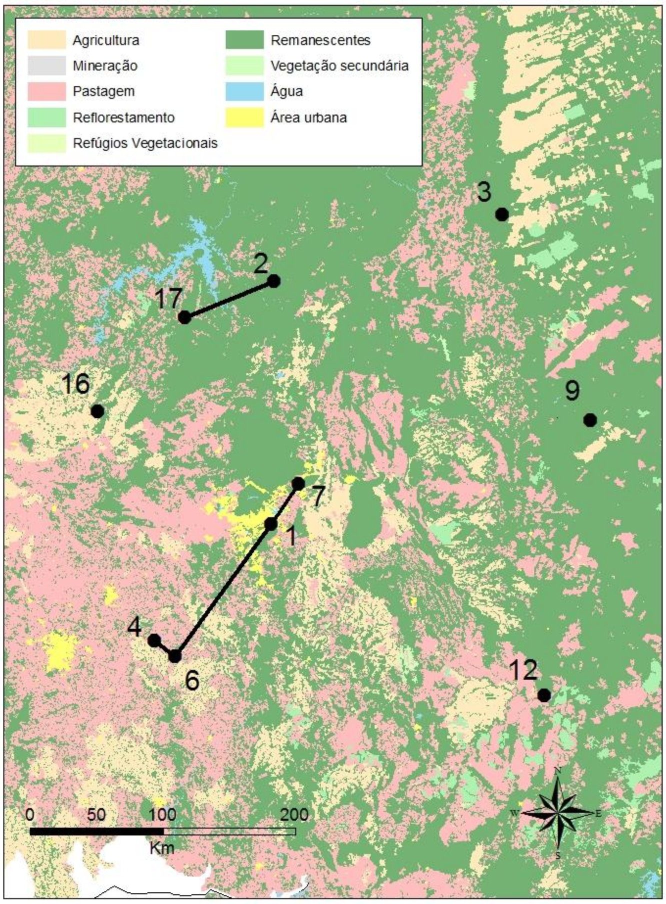

Figure 3 - Genetic discontinuity based on Delaunay's triangulation for 17 populations of *Glossophaga soricina* in Brazilian Cerrado Biome. Full lines indicate possible discontinuity between localities.

Using Maximum Likelihood Estimation model (mle2), variation in F_{IS} among populations was best explained by percentage of natural vegetation remnants at 2 km landscape-buffer (Table 3). Inbreeding was lower in areas with higher percentage of natural vegetation remnants (Figure 4). At 5 km landscape-buffer the null model explained better (Table 3). However, genetic diversity (H_e) and allelic richness (R_S) was explained equally by percentage of vegetation and the null model at both spatial scales (Table 3).

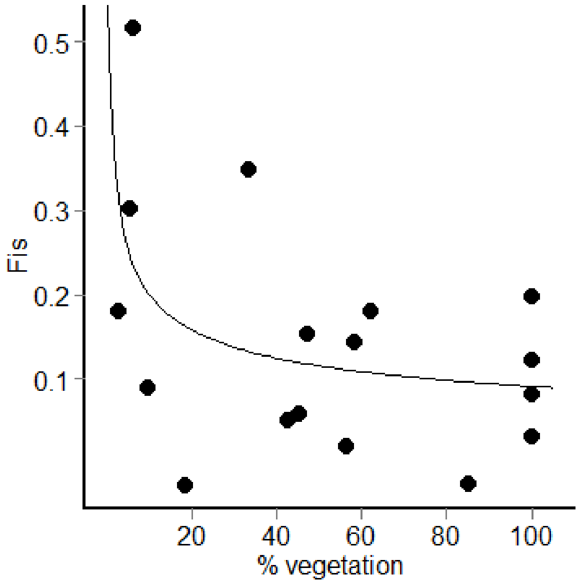

Figure 4 – Relationship between inbreeding coefficient (F_{IS}) and percentage of natural vegetation remnants in 17 populations of *Glossophaga soricina* in Brazilian Cerrado Biome.

Differentiation among population pairs (F_{ST}) was not related to any landscape features (global R^2 = 0.083, p = 0.735). Partial values for each parameter analyzed were also not significant (Table 4).

Table 3 – Model selection of the compete hypotheses to explain the pattern of variation in genetic diversity (H_E), allelic richness (R_S) and inbreeding coefficient (F_{IS}) in 17 populations of *Glossophaga soricina* in Brazilian Cerrado.

Model	**k**	**ΔAICc**	**wAIC**
2km- buffer			
H_E X % Natural vegetation remnants	**3**	**0.0**	**0.5292**
H_E Null model	**3**	**0.3**	**0.4606**
H_E X Matrix type	5	7.9	0.0102
F_{IS} X % Natural vegetation remnants	**3**	**0.0**	**0.88922**
F_{IS} Null model	3	4.3	0.10541
F_{IS} X Matrix type	5	10.2	0.00537
R_S X % Natural vegetation remnants	3	**0.0**	**0.6445**
R_S Null model	3	**1.3**	**0.3311**
R_S X Matrix type	5	6.5	0.0245
5 Km- buffer			
H_E Null model	3	**0.0**	**0.9604**
H_E X % Natural vegetation remnants	3	6.4	0.0394
H_E X Matrix type	6	17.2	<0.001
F_{IS} Null model	3	**0.0**	**0,83033**
F_{IS} X % Natural vegetation remnants	3	3.2	0,16785
F_{IS} X Matrix type	6	12.2	0,00182
R_S Null model	3	**0.0**	**0.88**
R_S X % Natural vegetation remnants	3	4.0	0.119
R_S X Matrix type	6	13.8	<0.001

K= number of parameters that consider the β of explanatory variables and the parameters of residuals distribution; AICc = AIC corrected by sample size and number of parameter in the model; wAIC = Akaike's weight of evidence.

Table 4 – Correlation between pairwise-F_{ST} and landscape features. R^2 = partial values for multiple Mantel tests.

Matrix feature	R^2	p-value
% Remnant vegetation	- 0.005	0.500
% Pastureland	0.018	0.543
% Agricultural area	- 0.002	0.605
% Urban area	- 0.061	0.494
Matrix resistance	0.0003	0.927

Discussion

Despite the high level of fragmentation and anthropogenic disturbs in Brazilian Cerrado, the studied populations of *G. soricina* presented relatively high genetic diversity, similar to the levels of other Phyllostomidae bats, such as *Lophostoma silviculum* (Dechmann et al. 2002), and *Carollia brevicauda* (Bardeleben et al. 2007). Notwithstanding, allelic richness was very low in all populations. However, genetic diversity and allelic richness was not explained by landscape features related to anthropogenic disturb, such as matrix type and percentage of natural vegetation remnants. This is most likely due to the recent fragmentation processes in Central Brazil (~60 years), especially for genetic diversity, which may take a few generations to be perceived (Keyghobadi 2007, Aguilar et al. 2008), even for species with short life cycles such as *G. soricina*. However, Ripperger et al (2012) demonstrated that land cover conditions explained the current genetic diversity in the bat species *Dermanura watsoni*, in a recently fragmented ecosystem in Costa Rica. We hypothesize that the lack of relationship of genetic diversity and allelic richness with landscape features related to fragmentation is due to the high vagility and versatility of *G. soricina* in habitat and food resources. This may also explain the lack of recent population size reduction signal.

Notwithstanding, our results showed that variation in inbreeding among populations could be explained by percentage of natural vegetation cover. Populations at localities with lower percentage of vegetation cover presented higher inbreeding coefficient. Thus, our results suggest that landscape may restrict *G. soricina* dispersal, increasing mating between closely related individuals. Indeed, Estrada et al. (1993) documented that bats avoid pasture's matrixes and Quesada et al. (2003) suggest that *G. soricina* is vulnerable to habitat disruption. However, our results also indicated that the relationship is scale dependent. In the larger scale (5 Km landscape-buffer) landscape features analyzed in the present study could not account for the variation in genetic parameters most likely because other variables may also be important, such as demographic history and colonization. Landscape ecology studies have also shown scale dependence in the relationship between habitat fragmentation and species occurrence and abundance (e.g. Gorresen et al. 2005, Boscolo and Metzger 2009). The differences observed between the landscape scales may also be related to *G. soricina* foraging behavior. Even though *G. soricina* is predominately nectarivorous, it can also consume other items, such as pollen, insects and fruits, if nectar is not available, requiring a smaller area to forage (Tschapka 2004, Lopez and Vaughan 2007).

Our results also showed low but significant differentiation (F_{ST} = 0.022) among populations of *G. soricina* in Brazilian Cerrado, and can be compared to other bat species (e.g. Salgueiro et al. 2008, Burland and Wilmer 2001). Comparison of F_{ST} and R_{ST} showed that allele sizes and stepwise mutation are informative to population differentiation (Hardy et al. 2003). These results may be the outcome of historical long distance dispersal and also due to the lack of sex-biased dispersal (Pink 1996). In fact, Bayesian cluster showed four genetic groups, even though the obtained pattern indicates weak genetic structure between sites with intense gene flow.

Notwithstanding, Bayesian cluster matched the genetic discontinuity results, despite the low genetic differentiation among populations. For instance, individuals from populations 1 and 6, 1 and 7, and 4 and 6 that showed a genetic discontinuity, where mostly clustered in different groups (Supplemental Information Table S4). This result may be duo to the recent fragmentation and anthropogenic disturbance because these areas are imbedded in urban area and pasture matrixes. These factors may affect the dispersal of *G. soricina* among localities, restricting gene flow and increasing inbreeding.

In conclusion, despite the high anthropogenic disturbance and fragmentation in the Central Brazil, *G. soricina* still presents high genetic diversity. However, our results showed that *G. soricina* populations have already higher inbreeding in fragmented landscapes in small geographic scales. Also, some pair of populations showed genetic discontinuity as the outcome of landscape modification. These findings are important for *G. soricina* conservation planning that should consider the local pattern of landscape fragmentation to recover natural vegetation, improving the connectivity among populations and potentially increasing dispersal.

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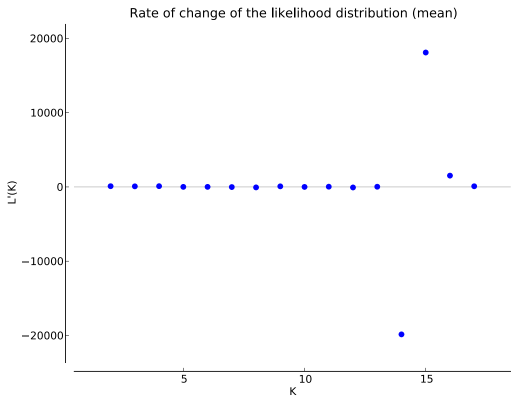
Figure S1 – Posterior probability graphs of the Bayesian clustering simulation implemented in the software Structure 2.3.4 (Pritchard et al. 2000)

(a)



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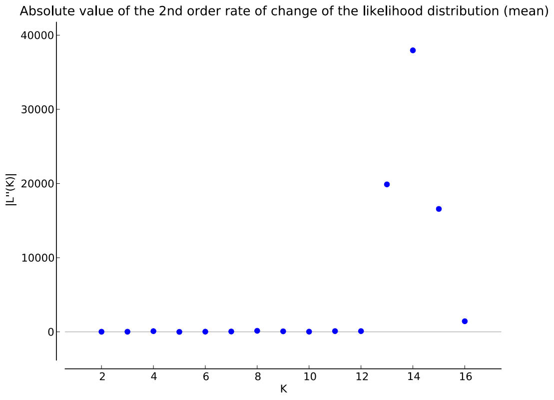

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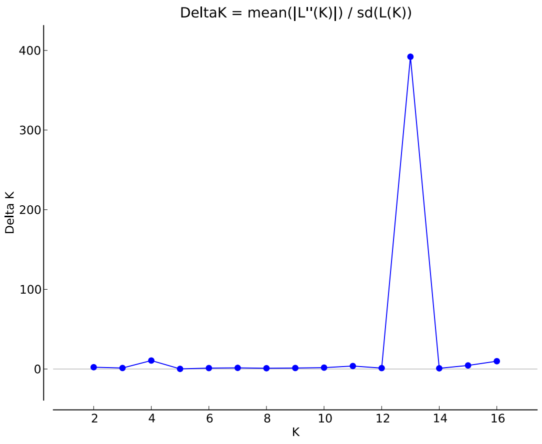
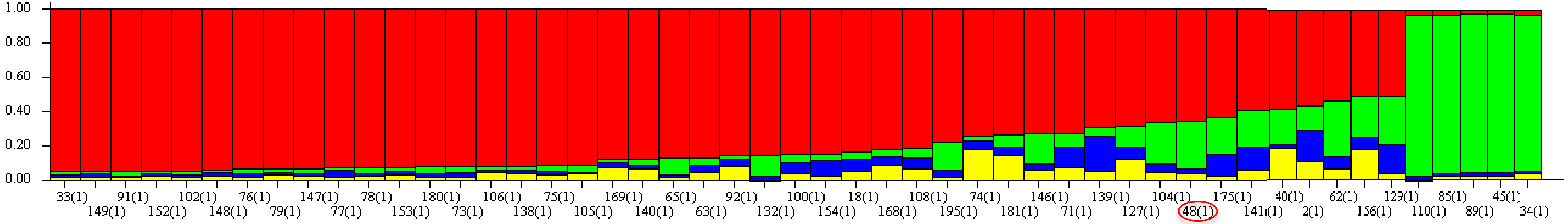
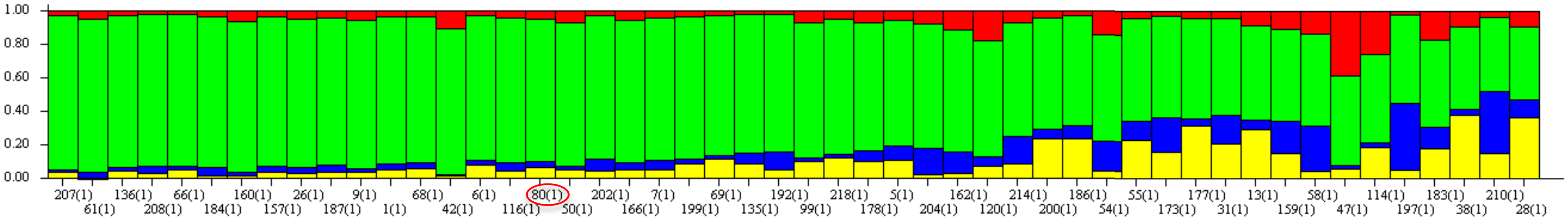


Figure S2 – Plots of Q-value (the estimated membership coefficients for each individual, in each cluster) for the individuals of 17 populations of *Glossophaga soricina* in Brazilian Cerrado Biome. Each individual in the data set is represented by a single vertical line, which is partitioned into K colored segments that represent that individual's estimated membership fraction in each of the K inferred clusters.

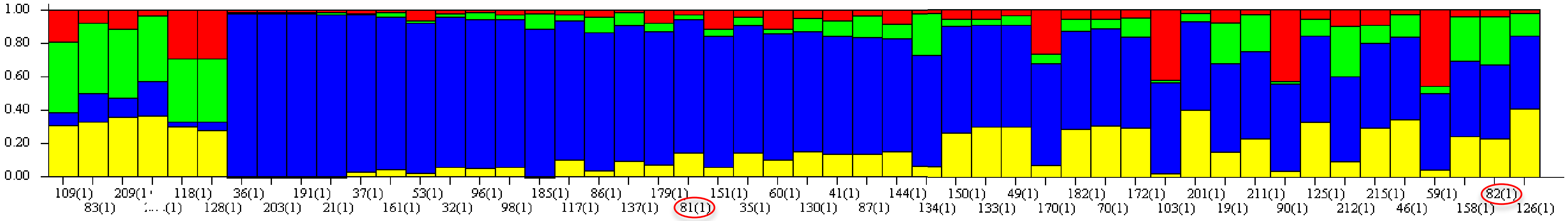
Group A



Group B



Group C



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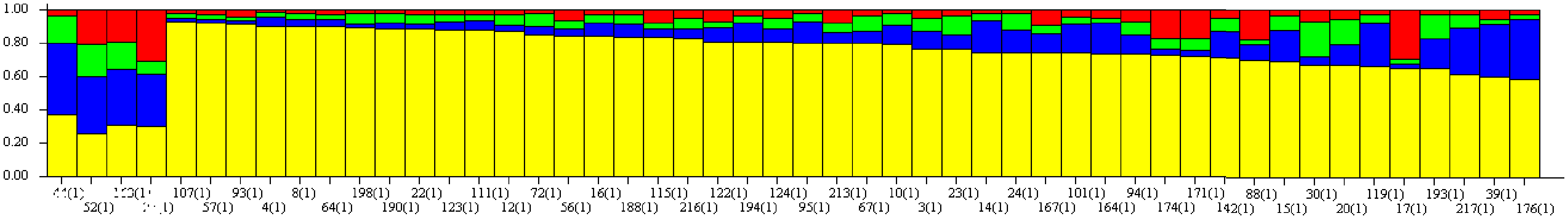


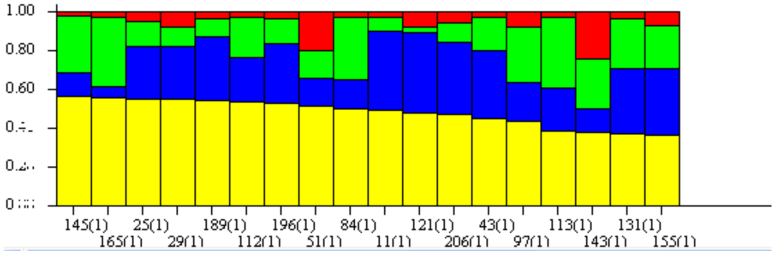
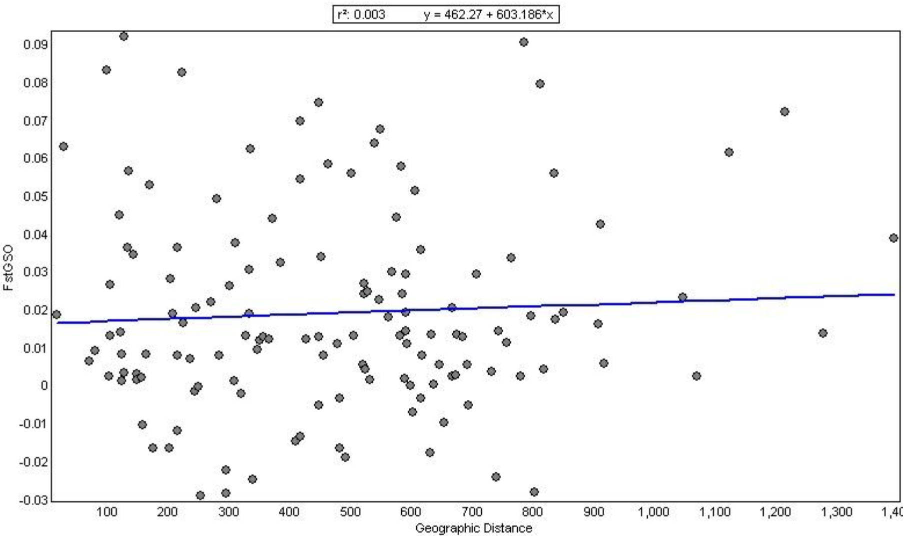


Figure S3 – (a) Correlation between the genetic distance (F_{ST}) and geographic distance in 17 populations of *Glossophaga soricina* in Brazilian Cerrado Biome; (b) Mantel correlogram.

(a)



(b)

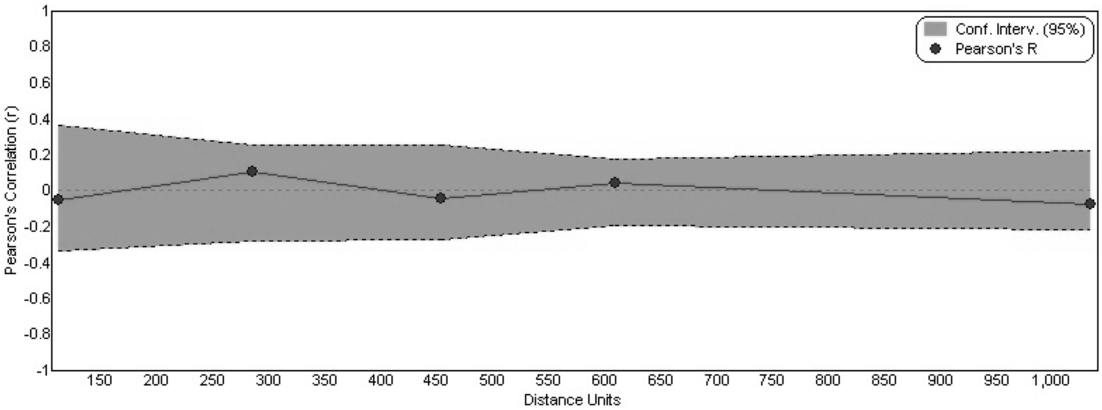


Table S1 – Location and number of individuals sampled in each subpopulation of *Glossophaga soricina* in Brazilian Cerrado Biome.

S	**Location**	**N**	**Latitude**	**Longitude**	**Matrix**
1	Brasília - DF	10	-47,861939	-15,855431	UA
2	P. N. Chapada dos Veadeiros - GO	22	-47,846806	-14,204583	NV
3	P. E. Terra Ronca - GO	10	-46,300000	-13,760000	NV
4	Floresta Nacional de Silvânia - GO	14	-48,651940	-16,640000	P
5	Dianápolis - Porto Franco - TO	15	-46,789300	-11,814300	NV
6	Vianópolis - GO	10	-4,851611	-16,748556	UA
7	E. E. Águas Emendadas - DF	7	-47,680000	-15,580000	AA
8	P. N. Chapada Diamantina - BA	10	-41,250000	-11,500000	P
9	P. N. Grande Sertão Veredas - MG	13	-45,700000	-15,150000	NV
10	P. N. Emas - GO	18	-52,998720	-17,901900	AA
11	Diamantina - MG	14	-43,388556	-18,271167	NV
12	Brasilândia de Minas - MG	9	-46,011111	-17,010278	UA
13	Nova Xavantina - MT	10	-52,501028	-14,835750	P
14	E. E. Itirapina - SP	9	-47,816700	-22,250000	UA
15	Pratânia - SP	8	-48,666111	-22,808333	UA
16	Barro Alto - GO	22	-49,040000	-15,086900	P
17	Niquelândia - GO	15	-48,450000	-14,450000	UA
	Total	216			

S = subpopulation; Matrix = predominant matrix in each buffer surrounding sampled location; NV = natural vegetation, UA = urban area, AA = agricultural area or P = pastureland

Table S2. Population subdivision indexes estimates (F_{IS}, F_{ST}, F_{IT} and R_{ST}) for *Glossophaga soricina* in the Brazilian Cerrado Biome, based in nine microsatellites markers.

Locus	F_{IS}	F_{ST}	F_{IT}	R_{ST}
GS 02	0.304	0.039	0.331	0.065
GS 03	0.150	0.005	0.154	-0.019
GS 04	0.309	0.028	0.328	0.021
GS 05	-0.107	0.009	-0.098	0.007
GS 10	-0.540	0.009	-0.525	0.007
GS 12	0.278	0.033	0.302	0.226
GS 13	0.330	0.025	0.346	0.007
GS 14	0.117	0.028	0.142	0.018
GS 15	0.320	0.026	0.337	0.071
Global	0.138	0.022	0.157	0.090

Table S3 – Pairwise F_{ST} for 17 subpopulations of *Glossophaga soricina* in the Brazilian Cerrado biome.

	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
1	0.063*	0.008	0.007	0.013*	0.083*	0.029*	0.034*	0.001	0.013	0.027*	0.079*	0.020*	0.001	0.016*	0.011	0.004*
2		0.026	0.019	0.036	0.092	-0.003	0.002	0.045*	0.026*	0.064*	0.090*	0.082*	0.024	0.036	0.056*	0.067*
3			-0.016	-0.016	0.028	-0.017	0.003	0.006	-0.011	0.005	0.033	-0.000	0.001	-0.022	-0.006	0.005
4				-0.024	0.030	-0.027	-0.002	-0.001	-0.010	-0.004	0.019*	0.003	-0.003	-0.028	0.002*	0.003*
5					0.018	-0.018	0.014	0.008	-0.013	-0.004	0.005	0.009	0.000	-0.028	0.012*	0.008
6						0.013	0.056	0.053	0.054	0.058	0.016	0.062	0.058	0.049	0.069*	0.074*
7							-0.016	0.018	0.004	0.008	0.039*	0.017*	0.002	0.002	-0.009	0.011
8								0.009	0.012	0.032*	0.042*	0.044*	0.013	0.012	0.022*	0.030*
9									0.022*	0.034*	0.056	0.037*	0.000	0.019	0.024*	0.008*
10										0.002	0.035*	0.008	0.013	-0.014	0.018*	0.019*
11											0.013	0.011	0.023	-0.024	0.014*	0.029*
12												0.044*	0.051	0.005	0.061*	0.072*
13													0.013*	0.001	0.014*	0.020*
14														0.001	0.013*	0.013
15															0.013*	0.024*
16																0.002

* = significant values ($p<0.05$)

Table S4 – Proportion of membership of each pre-defined population of *Glossophaga soricina* in each of the 4 clusters inferred by Bayesian analyses implemented in Structure software.

Individual	Original population	Inferred cluster			
		1	2	3	4
1	1	0.8774	0.0304	0.0568	0.3530
2	1	0.1619	0.5372	0.1026	0.1983
3	1	0.0789	0.0510	0.7591	0.1110
4	1	0.0240	0.0137	0.9148	0.0475
5	1	0.7262	0.0594	0.1156	0.0988
6	1	0.8623	0.0234	0.0782	0.0360
7	1	0.8288	0.0466	0.0562	0.0683
8	1	0.0379	0.0178	0.9041	0.0402
9	1	0.8673	0.0599	0.0448	0.0280
10	1	0.0743	0.0222	0.7767	0.1267
11	2	0.0835	0.0264	0.5051	0.3850
12	2	0.0645	0.0288	0.8613	0.0455
13	2	0.5706	0.0827	0.2887	0.0581
14	2	0.0449	0.0168	0.7770	0.1613
15	2	0.0886	0.0361	0.6835	0.1918
16	2	0.0481	0.0250	0.8574	0.0696
17	2	0.0246	0.2484	0.7030	0.0241
18	2	0.0522	0.8192	0.0560	0.0726
19	2	0.2386	0.0754	0.1457	0.5403
20	2	0.1315	0.0518	0.6950	0.1217
21	2	0.0156	0.0115	0.0167	0.9562
22	2	0.0458	0.0240	0.8912	0.0389
23	2	0.1220	0.0336	0.7571	0.0874
24	2	0.1067	0.0177	0.7611	0.1146
25	2	0.1270	0.0475	0.5716	0.2539
26	2	0.8820	0.0478	0.0348	0.0355
27	2	0.0823	0.3450	0.2747	0.2980
28	2	0.4247	0.0880	0.3733	0.1140
29	2	0.0989	0.0772	0.5627	0.2612
30	2	0.1931	0.0730	0.6865	0.0474
31	2	0.6038	0.0466	0.1943	0.1553
32	2	0.0284	0.0165	0.0650	0.8902
33	2	0.0169	0.9435	0.0193	0.0204
34	3	0.9193	0.0221	0.0401	0.0185
35	3	0.0526	0.0471	0.1513	0.7489
36	3	0.0082	0.0086	0.0103	0.9729
37	3	0.0128	0.0169	0.0319	0.9384
38	3	0.4815	0.0896	0.3907	0.0381
39	3	0.0302	0.0601	0.5971	0.3127

40	3	0.2023	0.5834	0.1897	0.0246
41	3	0.0781	0.0588	0.1099	0.7532
42	3	0.8828	0.0903	0.0156	0.0113
43	3	0.1606	0.0288	0.4413	0.3693
44	4	0.1465	0.0301	0.3585	0.4649
45	4	0.9265	0.0237	0.0217	0.0282
46	4	0.1370	0.0290	0.3151	0.5189
47	4	0.5256	0.3826	0.0667	0.0251
48	4	0.2798	0.6420	0.0399	0.0383
49	4	0.0609	0.0360	0.2621	0.6410
50	4	0.8419	0.0781	0.0600	0.0200
51	4	0.1255	0.1738	0.5609	0.1398
52	4	0.1847	0.2235	0.2585	0.3332
53	4	0.0165	0.0860	0.0248	0.8727
54	4	0.6108	0.1618	0.0480	0.1794
55	4	0.6116	0.0396	0.2100	0.1388
56	4	0.0541	0.0591	0.8463	0.0405
57	4	0.0226	0.0233	0.9281	0.0260
58	5	0.5216	0.1405	0.0414	0.2965
59	5	0.0458	0.4630	0.0421	0.4491
60	5	0.0249	0.1246	0.0928	0.7577
61	5	0.9108	0.0471	0.0160	0.0261
62	5	0.3179	0.5457	0.0628	0.0736
63	5	0.0471	0.8580	0.0499	0.0450
64	5	0.0315	0.0273	0.8955	0.0457
65	5	0.1128	0.8476	0.0235	0.0162
66	5	0.8978	0.0218	0.0579	0.0226
67	5	0.0992	0.0275	0.8091	0.0642
68	5	0.8612	0.0351	0.0700	0.0337
69	5	0.8262	0.0284	0.1197	0.0257
70	5	0.0567	0.0541	0.2914	0.5978
71	5	0.0853	0.7021	0.0856	0.1270
72	5	0.0744	0.0222	0.8450	0.0585
73	6	0.0512	0.9024	0.0208	0.0256
74	6	0.0285	0.7199	0.1929	0.0587
75	6	0.0390	0.8996	0.0332	0.0282
76	6	0.0275	0.9285	0.0200	0.0240
77	6	0.0139	0.9146	0.0231	0.0484
78	6	0.0413	0.9144	0.0246	0.0197
79	6	0.0239	0.9215	0.0360	0.0186
80	6	0.8447	0.0426	0.0822	0.0304
81	6	0.0308	0.0245	0.1495	0.7952
82	6	0.3014	0.0412	0.2015	0.4559
83	7	0.4102	0.0897	0.3130	0.1871
84	7	0.3537	0.0309	0.4476	0.1678
85	7	0.9301	0.0278	0.0239	0.0182

86	7	0.1027	0.0476	0.0448	0.8049
87	7	0.1508	0.0364	0.1491	0.6636
88	7	0.0267	0.1431	0.7467	0.0834
89	7	0.9306	0.0173	0.0288	0.0233
90	8	0.0181	0.4323	0.0416	0.5080
91	8	0.0281	0.9425	0.0177	0.0116
92	8	0.0230	0.8353	0.0922	0.0495
93	8	0.0247	0.0386	0.9151	0.0217
94	8	0.0697	0.0844	0.7360	0.1099
95	8	0.0568	0.0175	0.8176	0.1081
96	8	0.0440	0.0163	0.0538	0.8859
97	8	0.2582	0.0863	0.4601	0.1954
98	8	0.0362	0.0266	0.0633	0.8739
99	8	0.8012	0.0633	0.1135	0.0220
100	9	0.0481	0.8388	0.0430	0.0701
101	9	0.0526	0.0369	0.7347	0.1759
102	9	0.0234	0.9386	0.0178	0.0202
103	9	0.0166	0.4278	0.0254	0.5302
104	9	0.2555	0.6473	0.0468	0.0504
105	9	0.0421	0.9047	0.0378	0.0154
106	9	0.0211	0.9090	0.0491	0.0209
107	9	0.0355	0.0232	0.9166	0.0247
108	9	0.0627	0.7849	0.0834	0.0690
109	9	0.4124	0.1927	0.3076	0.0873
110	9	0.9476	0.0273	0.0139	0.0112
111	9	0.0407	0.0253	0.8778	0.0562
112	9	0.1992	0.0241	0.5450	0.2317
113	9	0.3445	0.0320	0.3880	0.2355
114	10	0.5259	0.2649	0.1784	0.0307
115	10	0.0400	0.0743	0.8286	0.0572
116	10	0.8555	0.0420	0.0479	0.0546
117	10	0.0346	0.0229	0.0866	0.8559
118	10	0.3567	0.2861	0.3314	0.0257
119	10	0.0587	0.0283	0.6322	0.2808
120	10	0.6779	0.1871	0.0784	0.0567
121	10	0.0318	0.0735	0.4569	0.4378
122	10	0.0348	0.0674	0.8103	0.0875
123	10	0.0515	0.0247	0.8693	0.0545
124	10	0.0714	0.0619	0.7762	0.0904
125	10	0.1023	0.0640	0.3080	0.5257
126	10	0.1302	0.0178	0.4158	0.4362
127	10	0.1185	0.6632	0.1439	0.0744
128	10	0.3980	0.2679	0.2818	0.0523
129	10	0.2834	0.5221	0.0412	0.1532
130	10	0.0809	0.0581	0.1681	0.6930
131	10	0.2384	0.0336	0.3491	0.3789

132	11	0.1411	0.8318	0.0131	0.0140
133	11	0.0368	0.0605	0.2789	0.6238
134	11	0.2385	0.0178	0.0574	0.6864
135	11	0.8248	0.0203	0.0821	0.0728
136	11	0.9017	0.0284	0.0430	0.0269
137	11	0.0724	0.0152	0.0604	0.8521
138	11	0.0224	0.9112	0.0400	0.0265
139	11	0.0534	0.6744	0.0561	0.2162
140	11	0.0408	0.8557	0.0781	0.0255
141	11	0.2074	0.5773	0.0675	0.1478
142	11	0.0820	0.0425	0.7291	0.1465
143	11	0.2586	0.2608	0.3454	0.1352
144	11	0.0969	0.0955	0.1264	0.6812
145	11	0.3163	0.0172	0.5353	0.1312
146	12	0.2024	0.6977	0.0625	0.0374
147	12	0.0359	0.9250	0.0249	0.0141
148	12	0.0211	0.9320	0.0285	0.0184
149	12	0.0172	0.9430	0.0221	0.0178
150	12	0.0475	0.0573	0.2696	0.6256
151	12	0.0458	0.1316	0.0644	0.7581
152	12	0.0172	0.9395	0.0245	0.0188
153	12	0.0237	0.9137	0.0345	0.0281
154	12	0.0330	0.8570	0.0292	0.0808
155	13	0.2052	0.0742	0.3246	0.3961
156	13	0.2096	0.5486	0.1765	0.0653
157	13	0.8961	0.0317	0.0393	0.0329
158	13	0.2469	0.0431	0.2278	0.4822
159	13	0.5261	0.1203	0.1401	0.2135
160	13	0.8978	0.0605	0.0187	0.0230
161	13	0.0288	0.0157	0.0482	0.9073
162	13	0.7119	0.1172	0.0386	0.1323
163	13	0.1564	0.1967	0.3101	0.3368
164	13	0.0357	0.0554	0.7194	0.1896
165	14	0.3726	0.0233	0.5491	0.0550
166	14	0.8480	0.0551	0.0538	0.0430
167	14	0.0509	0.0999	0.7310	0.1181
168	14	0.0423	0.8127	0.0917	0.0533
169	14	0.0207	0.8685	0.0828	0.0280
170	14	0.0560	0.2831	0.0763	0.5845
171	14	0.0695	0.1621	0.7255	0.0429
172	14	0.1086	0.0516	0.2436	0.5963
173	14	0.6001	0.0280	0.1534	0.2185
174	15	0.0564	0.1395	0.7705	0.0336
175	15	0.2312	0.6094	0.0228	0.1366
176	15	0.0342	0.0264	0.5976	0.3419
177	15	0.5870	0.0479	0.3188	0.0464

178	15	0.7698	0.0634	0.1014	0.0654
179	15	0.0513	0.0897	0.0825	0.7765
180	15	0.0383	0.9195	0.0187	0.0234
181	15	0.0673	0.7149	0.1619	0.0559
182	16	0.0764	0.0552	0.2753	0.5932
183	16	0.5223	0.1685	0.1781	0.1311
184	16	0.8917	0.0288	0.0181	0.0614
185	16	0.0939	0.0241	0.0145	0.8675
186	16	0.6665	0.0255	0.2207	0.0873
187	16	0.8658	0.0416	0.0439	0.0487
188	16	0.0533	0.0322	0.8401	0.0744
189	16	0.1014	0.0342	0.5096	0.3547
190	16	0.0570	0.0218	0.8832	0.0380
191	16	0.0095	0.0102	0.0153	0.9650
192	16	0.8187	0.0198	0.0535	0.1079
193	16	0.1611	0.0246	0.6460	0.1683
194	16	0.0481	0.0297	0.7963	0.1258
195	16	0.1782	0.7555	0.0220	0.0443
196	16	0.1435	0.0401	0.4934	0.3231
197	16	0.5229	0.0183	0.0492	0.4096
198	16	0.0647	0.0203	0.8880	0.0270
199	16	0.8371	0.0316	0.0940	0.0373
200	16	0.6751	0.0449	0.2167	0.0633
201	16	0.0502	0.0183	0.3890	0.5425
202	16	0.8449	0.0299	0.0425	0.0826
203	16	0.0093	0.0089	0.0139	0.9679
204	17	0.7270	0.0762	0.0244	0.1725
205	17	0.3692	0.0368	0.4045	0.1896
206	17	0.1080	0.0584	0.4141	0.4195
207	17	0.9163	0.0238	0.0391	0.0207
208	17	0.9020	0.0236	0.0350	0.0394
209	17	0.3923	0.1216	0.3772	0.1089
210	17	0.4127	0.0350	0.1576	0.3947
211	17	0.2298	0.0252	0.2074	0.5377
212	17	0.0592	0.0854	0.7909	0.0646
213	17	0.6653	0.0740	0.0758	0.1849
214	17	0.1092	0.0908	0.2494	0.5506
215	17	0.0853	0.0286	0.6101	0.2760
216	17	0.8003	0.0488	0.1306	0.0202

Table S5 - Probability of heterozygosity excess for the Wilcoxon test implemented in the Bottleneck software, for the three mutation models. IAM, infinite allele mutation model; SMM, stepwise mutation model; TPM two-phase mutation model, with 70% of SMM.

Population	**IAM**	**SMM**	**TPM**
A	0.00684	0.98145	0.17969
B	0.21289	0.99023	0.82031
C	0.45508	0.99023	0.87500
D	0.21289	0.89844	0.45508