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Population Variability of Exotic and Native Blowflies in Brazil, Based on Mitochondrial DNA Sequences

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ABSTRACT

A molecular phylogeny analysis was performed on blowfly species. Molecular analyses entailed the comparative sequence analysis of the cytochrome oxidase subunit I (COI) DNA, amplified from individuals by means of the polymerase chain reaction (PCR). The 310 base pairs of the mitochondrial COI sequences analysis were analysed, and revealed the existence of 235 invariant sites and 75 polymorphic sites, with 71 parsimony informative sites. Invariant positions in the sequence were removed, and the remaining variant positions in the sequence indicated the number of substitutions supporting the divergence of the taxa. The gene analyses revealed the existence of different haplotypes in *Chrysomya albiceps*, *Cochliomyia macellaria*, and *Lucilia eximia*. Phylogenetic analyses through tree topology showed the existence of well-defined mitochondrial lineages among exotic and native blowflies. Seven distinct congeneric clusters were formed based on the sequence data. The results are discussed in genetic and ecological contexts.

Keywords: COI, Diptera, genetic variability, molecular analyses, mtDNA

INTRODUCTION

The diversity and abundance of blowflies in South America has been changing over the last 30 years, principally in response to introduction of exotic species of the genus *Chrysomya* (Guimarães *et al*. 1978, 1979; Baumgartner and Greenberg 1984). Phenomena such as this demand more effort in terms of systematics to increase knowledge of blowfly diversity, principally in areas where the previous diversity of flies was high. Most entomological evidence is strongly dependent on accurate species identification. Identification of individuals may be complicated by many factors, including the diversity of adult fly species, the particular larval life stage collected, and the collection of dead insects only (Wallman and Donnellan 2001). Molecular data are helpful in identifying insect specimens, especially when no specimen in suitable condition for morphological identification is obtained.

Molecular analysis is also useful to analyse population profiles, principally in comparative studies, which investigate the taxonomic status in a biological invasion context. The introduction of exotic blowfly species into the Americas created an interesting scenario, as pointed out by Wells and Sperling (1999). They emphasised that if on the one hand, there is experimental evidence showing that calliphorid species such as *L. cuprina* and *L. sericata*, or *C. megacephala* and *C. pacifica* can produce fertile hybrids (Wells and Sperling 1999), on the other hand genetic variation in populations of *L. cuprina* and *L. sericata* is also possible (Stevens and Wall 1997).

Wells and Sperling (1999) used mtDNA to infer the molecular-phylogenetic relationships of *C. albiceps* and *C. rufifacies* from widely separated localities in the Old and New World. Several other studies have attempted to address these issues in calliphorid Diptera by using mitochondrial DNA as the basis for sequencing (Malgorn and Coquoz 1999; Wallman and Donnellan 2001; Harvey *et al*. 2003). In this study we also used this technique, however to perform analyses from localities in Brazil that are separated by only short distances. Previous studies have suggested that genetic differentiation is possible in Diptera even over short distances (Lehnmann *et al*. 2003).

Most literature focused on necrophagous insects has addressed the fauna of Australia, Europe, and the United States. In South America, particularly in Brazil, necrophagous fauna has gradually received more attention (Souza and Linhares 1997; Carvalho and Linhares 2001). Until now, Brazilian researchers have focused on the succession of insects on animal carcasses (Carvalho *et al*. 2000; Carvalho and Linhares 2001). Despite increasing interest in forensic entomology, DNA-based identification still remains a line to be pursued in Brazil. This is a result of the small amount of genetic data collected on the current Brazilian blowfly fauna, with emphasis on comparisons between native and introduced species. However, its usefulness has become evident, as several African insect species have been observed in South America (Guimarães *et al*. 1978, 1979; Baumgartner and Greenberg 1984). Then, the objective of this study was to perform a molecular phylogeny analysis on blowfly species in Brazil, in order to investigate their population profiles.

MATERIALS AND METHODS

We sequenced mitochondrial cytochrome oxidase subunit I (COI) DNA of six blowfly species, to study its usefulness for their differentiation. The work reported here used both morphological and molecular approaches to study specimens from four geographical regions. Morphological analyses were made on adults, using all of the external characters, such as metallic color, stripes, spiracle, gena, spines, bristles and hairs that had been identified (Guimarães and Papavero 1999) previously as being of value in separating geographical races. Molecular analyses entailed the comparative sequence analysis of the cytochrome oxidase subunit I (COI) DNA, amplified from individual flies using the polymerase chain reaction (PCR). Previous studies in molecular phylogenetics of medically important Diptera had indicated that this segment of the maternally inherited mtDNA is a suitable source for markers to study geographical variation (Ready *et al*. 1997; Esseghir *et al*. 2000), in part because mtDNA rarely recombines and has a relatively rapid rate of nucleotide substitution (Avise 1994).

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Flies and materials

Specimens of *Chrysomya albiceps* (31 individuals), *C. megacephala* (25 individuals), *C. putoria* (3 individuals), *Lucilia eximia* (10 individuals), *L. cuprina* (7 individuals), *Cochliomya macellaria* (5 individuals), and *Hemilucilia segmentaria* (9 individuals) were used in this study and collected in four areas, in the cities of Nova Andradina in the state of Mato Grosso do Sul (22°14′00″ South, 53°20′35″ West), placed at a distance of 610 km from Botucatu, 999 km from Gramado and 242 km from Presidente Prudente, in Gramado in the state of Rio Grande do Sul (29°24′17″ South, 50°52′35″ West) placed at a distance of 914 km from Botucatu and 970 km from Presidente Prudente. Flies also were collected in the city of Presidente Prudente, state of São Paulo (22°07′ 32″ South, 51°23′23″ West), placed at a distance of 377 km from Botucatu and in the city of Botucatu in the state of São Paulo (22° 53′09″ South, 48°26'42" West), all in Brazil (**Table 1**). In the first three areas, the flies were collected in municipal garbage by using baits. Traps were set with plastic drinking bottles (2000 ml, 9 cm diameter × 30 cm length), each of them with a hole in its bottom and chicken viscera placed inside.

The characters used in morphological identification were the prothoracic spiracle and the postsutural achrostical bristles (Guimarães and Papavero 1999). A Zeiss Stemi 2000 (W_Pl 10x/23) was used to observe the characters. A data matrix was prepared for each specimen.

Genome DNA extraction

Total individual DNA was extracted from individual female flies (90 individuals corresponding to six species), and amplified by PCR based on the universal primers C1-J-2495 (5′-CAGCTACT TTATGAGCTTTAGG-3′) and C1-N-2800 (5′-CATTTCAAGT/CT GTGTAAGCATC-3′) reported by Wells and Sperling (1999). Each specimen was preserved in 70% ethanol.

PCR

The amplification reaction was carried out in a total volume of 25 µl, with a final concentration of 1 × PCR buffer (Tris-HCl 20 mM pH 8.4; KCl 50 mM), 1.5 mM $MgCl_2$, 0.2 mM DNTPs (Invitrogen), 1U of Taq DNA polymerase (Pharmacia) and 1 µM of each of the primers. One microlitre portion of the DNA extract was used for PCR amplification.

The PCR reactions were performed with the thermal profile consisting of a hot start of 2 repetitive cycles of 2 min at 94ºC, 2 min at 37ºC, and 1 min at 72ºC followed by 35 repetitive cycles of 30 s at 94ºC, 30 s at 50ºC, and 1 min at 72ºC, followed by an additional extension cycle at 72ºC for 5 min. All amplifications were performed on a Whatman Biometra® (T gradient) thermocycler.

Aliquots of amplified products (8 µl) were analysed by running a 1% agarose electrophoresis containing ethidium bromide (0.5 mg/ml) and visualised under ultraviolet illumination. A low DNA mass ladder was used as a base-pair molecular weight pattern (Low DNA MASS Ladder, Invitrogen). The total remaining reaction products were purified by purification Kit "QIAquick® PCR Purification - Qiagen".

Sequence

Sequencing of PCR products amplified from fly samples was carried out in both directions using the "ABI Prism® Big Dye^M Terminator Cycle Sequencing Ready Reaction Kit" (PE Applied Biosystems, Forter City, California, USA). Approximately 10 ng of purified DNA, for each sequencing reaction, was combined with 3.2 ρmol of primer (sense and/or reverse) used in the amplification reaction. Nucleic acid sequence analysis was performed on an automated Applied Biosystems 377 DNA sequence.

Sequence analysis

The computer analysis of 310 base pairs of the mitochondrial COI haplotypes was performed using MERGER (http://bioweb.pasteur.fr/seqanal/alignment/intro-uk.html) package software to produce a consensus sequence for each DNA sample used.

The nucleotide sequences of the five species were aligned using Clustal W software (Thompson *et al*. 1994) set to default parameters, with manual adjustments where necessary. Aligned sequences were analysed using the MEGA software package (Kumar *et al*. 1994). Methods of Distance (Neighbour-Joining – NJ) and Parsimony were used to construct the phylogenetic tree (Saitou and Nei 1987). A phylogenetic tree was visualised using the TREE VIEW 1.4 program (Page 1996). The bootstrap test was applied to estimate the confidence of branching patterns of the neighbour-joining tree (Felsenstein 1985).

Statistical analysis of mitochondrial haplotype frequencies

For each collection, the nucleotide sequence and frequency of each haplotype were entered into DnaSP v 3.5 (Rozas and Rozas 1999). We estimated the number of polymorphic sites, the average number of nucleotide differences (k), the nucleotide diversity (π_1), the diversity with jukes and cantor correction (π_2), the synonymous and nonsynonymous sites, and haplotype diversity (*Hd*).

GenBank accession numbers

The nucleotide sequences reported in this paper have the following GenBank accession numbers: CALB1 EF136633, CALB2 EF1 36634, LEXI3 EF136635, LEXI1 EF136636, LEXI4 EF136637, LEXI2 EF136638, LEXI5 EF136639, LCUP EF136640, CMEG EF136641, CPUT EF136642, CMAC1 EF136643, CMAC3 EF1 36644, CMAC4 EF136645, CMAC2 EF136646 and HLUC EF1 36647.

RESULTS

Sequences of 90 individual calliphorid flies were successfully sequenced and aligned (**Table 1**). The 310 base pairs of the mitochondrial COI sequences analysis were analysed, and revealed the existence of 235 invariant sites and 75 polymorphic sites with 71 parsimony informative sites. Invariant positions in the sequence were removed, and the remaining variant positions in the sequence indicated the number of substitutions supporting the divergence of the taxa (**Table 2**).

The gene analyses revealed the existence of two different haplotypes in *C. albiceps*, four haplotypes in *C. macellaria*, and five haplotypes in *L. eximia*. All the other populations showed only one haplotype. The number of variable loci and the observed frequencies for each collection and for all species are shown in **Table 3**. Collections had an average number of nucleotide differences among individuals of $\kappa = 22.895$ with the nucleotide diversity $\pi = 0.7386$.

Phylogenetic analyses through tree topology, which gave identical results as neighbour-joining and maximum parsimony methods, showed the existence of well-defined mitochondrial lineages defined among exotic and native blowflies. Seven distinct congeneric clusters were formed based on the sequence data. High bootstrap values supported the three nodes. Bootstrap values provide an indication

Table 1 Locations and sample sizes of blowfly collections in Brazil.

Region	Specimens
Botucatu, SP	Lexi: 10; Lcup: 7; Cmeg: 15; Calb: 14; Hluc: 9; Cmac: 5.
Presidente Prudente, SP	Cput: 3; Cmeg: 6.
Nova Andradina, MS	Cmeg: 2; Calb: 17.
Gramado, RS	Cmeg: 2.

Lexi: *L. eximia*; Lcup: *L. cuprina*; Cmeg: *C. megacephala*; Calb: *C. albiceps*; Hluc: *Hemilucilia*; Cmac: *C. macellaria*; Cput: *C. putoria*

Table 2 Nucleotide substitutions for variant positions in partial sequences obtained for *H. segmentaria*, *C. macellaria*, *C. putoria*, *L. cuprina*, *C. megacephala*, *C. albiceps*, and *L. eximia* numbered relative to the entire sequence.

	4	**7**	**13**	**19**	**22**	**28**	**31**	**37**	**40**	**43**	**52**	**55**	**58**	**61**	**62**	**67**	**73**	**74**	**76**	**82**	**85**	**88**	**97**	**103**	**109**
H. segmentaria	A	T	C	C	T	A	A	T	A	T	A	T	T	A	A	T	T	C	A	T	T	T	T	T	C
C. macellaria 1	.	C	.	.	A	.	.	.	T	.	T	C	A	.	.	.	C	T	.	.	.	.	A	C	.
C. macellaria 2	.	C	.	.	A	.	.	.	T	.	.	C	A	.	.	.	C	T	.	.	.	.	A	C	.
C. macellaria 3	G	C	.	.	A	.	.	.	T	.	.	C	A	.	.	.	C	T	.	.	.	.	A	C	.
C. macellaria 4	G	C	.	.	A	.	.	.	T	.	.	C	A	.	.	.	C	T	.	.	.	.	A	C	.
C. putoria	.	.	T	T	.	.	.	.	.	.	.	.	.	.	G	.	.	.	T	C	A	.	A	.	.
C. megacephala	.	.	T	T	.	.	.	.	.	.	.	.	A	.	.	C	.	T	.	.	A	.	A	C	T
L. cuprina	.	.	T	.	.	.	T	.	.	.	.	C	.	.	G	.	.	T	.	.	A	C	A	C	T
C. albiceps 1	.	.	T	T	.	.	.	.	.	.	.	.	A	T	.	.	.	T	.	.	A	.	A	C	T
C. albiceps 2	.	.	T	T	.	.	.	.	.	.	.	.	A	T	.	.	.	T	.	.	A	.	A	C	T
L. eximia 3	.	.	T	T	.	.	.	A	.	A	.	C	.	T	G	.	.	T	.	.	A	C	A	.	.
L. eximia 4	.	.	T	T	.	.	.	A	.	A	.	C	.	T	G	.	.	T	.	.	A	C	A	.	.
L. eximia 5	.	.	T	T	.	.	.	A	.	A	.	C	.	T	G	.	.	T	.	C	A	C	A	.	.
L. eximia 1	.	.	T	T	.	G	.	A	.	A	.	C	.	T	G	.	.	T	.	C	A	.	A	.	.
L. eximia 2	.	.	T	T	.	G	.	A	.	A	.	C	.	T	G	.	.	T	.	C	A	.	A	.	.

	115	**116**	**118**	**121**	**124**	**130**	**133**	**136**	**148**	**151**	**154**	**157**	**160**	**165**	**166**	**169**	**170**	**175**	**178**	**182**	**187**	**188**	**193**	**194**	**195**
H. segmentaria	A	C	A	T	A	A	A	T	T	A	C	T	C	A	C	T	C	T	A	C	C	T	T	A	G
C. macellaria 1	.	T	.	.	.	.	.	.	A	.	T	A	C	.	T	.	.	.	.	T	T	.	.	.	.
C. macellaria 2	.	T	.	.	.	.	.	.	A	.	T	A	C	.	T	.	.	.	.	T	T	.	.	.	.
C. macellaria 3	.	T	.	.	.	.	.	.	A	.	T	A	C	.	T	.	.	.	.	T	T	.	.	.	.
C. macellaria 4	.	T	.	.	.	.	.	.	A	.	T	A	C	.	.	.	.	.	.	T	T	.	.	.	.
C. putoria	.	T	.	A	.	T	.	C	A	G	T	.	T	T	.	.	.	.	T	T	T	C	.	G	.
C. megacephala	T	.	.	A	G	T	.	.	A	.	T	.	T	T	.	.	.	.	T	T	T	.	.	.	.
L. cuprina	T	T	.	A	.	T	.	.	A	.	T	.	.	.	.	.	T	C	.	T	T	.	.	.	C
C. albiceps 1	T	.	T	A	.	T	.	C	A	.	.	.	T	T	.	A	T	.	T	T	T	C	.	.	A
C. albiceps 2	T	.	T	A	.	T	.	C	A	.	.	.	T	T	.	A	T	.	T	T	T	C	.	.	A
L. eximia 3	.	T	.	A	.	.	T	C	A	.	T	C	T	.	.	A	T	.	.	T	.	.	C	.	C
L. eximia 4	.	T	.	A	.	.	T	C	A	.	T	C	T	.	.	A	T	.	.	T	.	.	C	.	C
L. eximia 5	.	T	.	A	.	.	T	C	A	.	T	C	T	.	.	A	T	.	.	T	.	.	C	.	C
L. eximia 1	.	T	.	A	.	.	T	C	A	.	T	C	T	.	T	A	T	.	.	T	.	.	C	.	C
L. eximia 2	.	T	.	A	.	.	T	C	A	.	T	C	T	.	T	A	T	.	.	T	.	.	C	.	C

	196	**199**	**200**	**203**	**208**	**217**	**218**	**232**	**235**	**242**	**247**	**250**	**253**	**262**	**265**	**274**	**277**	**280**	**288**	**291**	**295**	**301**	**304**
H. segmentaria	A	G	A	C	A	C	G	T	A	T	T	C	C	T	C	A	G	A	G	G	C	C	T
C. macellaria 1	.	.	T	T	G	T	.	.	G	.	.	.	.	C	.	.	A	T	.	.	.	T	.
C. macellaria 2	.	.	T	T	G	T	.	.	G	.	.	.	.	C	.	.	A	T	.	.	.	T	C
C. macellaria 3	.	.	T	T	G	T	.	.	G	.	.	.	.	C	.	.	A	T	.	.	.	T	C
C. macellaria 4	.	.	T	T	G	T	.	.	G	.	.	.	.	C	.	.	A	T	.	.	.	T	C
C. putoria	T	.	.	T	G	T	.	.	.	.	A	.	.	.	.	.	A	.	.	.	T	.	.
C. megacephala	C	.	T	T	G	T	.	C	.	.	A	.	.	.	.	.	A	T	.	.	.	.	.
L. cuprina	T	.	.	T	.	T	.	.	G	.	A	.	.	.	.	.	A	.	.	.	.	.	.
C. albiceps 1	T	A	.	T	.	T	.	.	.	.	A	.	T	.	T	.	T	T	.	.	T	.	C
C. albiceps 2	T	A	.	.	.	T	.	.	.	.	A	.	T	.	T	.	T	T	.	T	T	.	C
L. eximia 3	.	A	.	T	.	.	A	.	G	C	A	T	.	C	.	G	C	.	.	.	.	T	.
L. eximia 4	.	A	.	T	.	.	A	.	.	C	A	T	.	C	.	G	C	.	.	.	.	T	.
L. eximia 5	.	A	.	T	.	.	A	.	.	C	A	T	.	C	.	G	C	.	.	.	.	T	.
L. eximia 1	.	A	.	T	.	.	A	.	.	C	A	T	.	C	.	G	C	.	T	.	.	.	.
L. eximia 2	.	A	.	T	.	.	A	.	.	C	A	T	.	C	.	G	C	.	.	.	.	.	.

Table 3 The sequences segregating sites, haplotypes, haplotype diversity (*Hd*), average number of nucleotide differences (*k*) nucleotide diversity (π) of blowfly collections in Brazil.

	Sequences	**Segregating sites**	**Haplotypes**	**Hd**	**κ**	**π**
C. albiceps	31	4	2	0,488	0,488	0,00157
L. eximia	10	7	5	0,8000	2,97778	0,00961
L. cuprina	7	0	1	0	0	0
C. megacephala	25	0	1	0	0	0
C. putoria	3	0	1	0	0	0
C. macellaria	5	3	4	0,9000	1,6000	0,00516
H. lucilia	9	0	1	0	0	0

of the percentage support for a grouping by randomly re-sampling the data.

The three species of the genus *Chrysomya* were grouped with high bootstrap support. At the species level, specimens of *C. macellaria* and *L. eximia* formed single clusters with 100% support (**Fig. 1**). Within the *L. eximia* clade considerable variation was evident, showing two other clusters with high support (**Fig. 1**). The long-branch lengths supported the division between the two groups.

DISCUSSION

The high support for the congeneric grouping of species illustrates the potential of the COI for use in interspecific distinction. The ability to clearly distinguish among these five forensically prominent genera based on such a small region provides a strong indication of the possible utility of using a larger region of the COI.

There are many questions concerning the ecological and evolutionary behaviour of blowfly species that could be elucidated using information from molecular markers. The

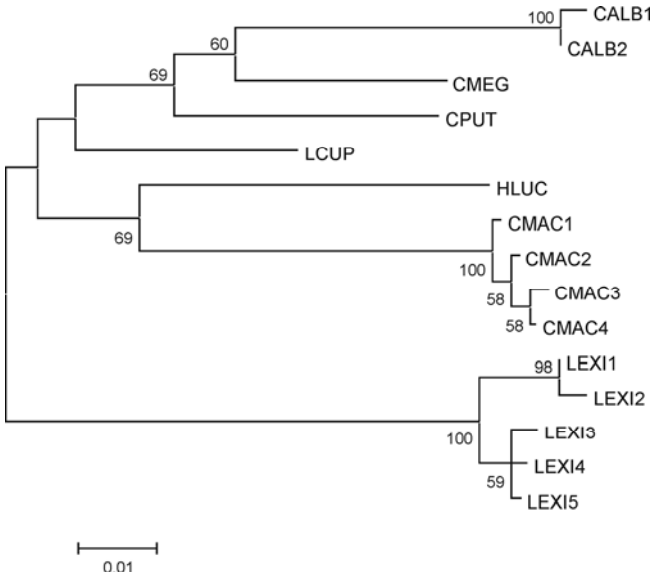

Fig. 1 Blowfly phylogeny based on Neighbour-Joining genetic distances among populations in Brazil. Bootstrap values over 50%, based on 1,000 permutations, are indicated on the nodes.

colonisation of the Americas by *Chrysomya* species has reportedly led to reduction in the native fly fauna (Prado and Guimarães 1982; Baumgartner and Greenberg 1984). The decrease in the genetic variability of *C. macellaria* populations has been associated with the presence of *Chrysomya* (Valle and Azeredo-Espin 1995). However, the exact source of New World *Chrysomya* remains to be defined.

Chrysomya albiceps and *C. rufifaces* are recognised as being difficult to distinguish morphologically (Tantawi and Greenberg 1993; Stevens and Wall 1997; Wells and Sperling 1999). The controversial taxonomic status of *C. albiceps* and *C. rufifacies* has recently been investigated using mtDNA markers, which provide an unambiguous approach to species identification (Wells and Sperling 1999, 2001). In Latin America, where the distributions of these species overlap (Tantawi and Greenberg 1993), the investigation of useful mitochondrial and nuclear DNA markers may be important for ecological, forensic, and genetic studies.

Separation of all seven species *C. albiceps*, *C. megacephala*, *C. putoria*, *L. cuprina*, *C. macellaria*, *H. segmentaria*, and *L. eximia* was highly supported; with high bootstrap values supporting the nodes, marking this region as useful for identification of these species.

The recent reports of primary myiasis caused by *L. eximia* in Brazil (Azeredo-Espin and Madeira 1996; Moretti and Thyssen 2006) suggest that it would be important to investigate the evolutionary processes related to these facultative species. Stevens *et al*. (2002) demonstrated divergent nuclear and mitochondrial phylogenies in hybrid *Lucilia* spp. Given the apparently great age of these subfamilies (Chrysomynae and Luciliinae), and by definition the lineages within them, it is perhaps not surprising that some minor variation in the intra-subfamily relationships defined by such diverse genes (nuclear/non-protein coding versus mitochondrial/protein coding) should occur (Gaunt and Miles 2002).

In addition, *L. eximia* has interesting behavioural differences at the individual and population levels compared to other calliphorid species. It is frequently found in rural and urban areas, and breeds primarily in carcasses but also in rotten fruit and urban garbage (Prado and Guimarães 1982; Madeira *et al*. 1989) and has been reared from a wide variety of corpses, including pigs (Souza and Linhares 1997).

Introduced and native blowfly species have shown interesting differences in terms of dynamic behaviour in Brazilian populations (Godoy *et al*. 2001). A research programme was initiated 12 years ago in order to understand the process of invasion by blowflies in Brazil (Godoy *et al*. 1996; Reis *et al*. 1996; Godoy *et al*. 1997, 2001; Silva *et al*. 2003). In this programme, mathematical and biological approaches have been integrated in order to address questions involving spatio-temporal dynamics. Using the Prout and McChesney model (Prout and McChesney 1985) which considers fecundity and survival as functions of immature density, the dynamic behaviour of *C. megacephala*, *C. putoria*, *C. albiceps*, *C. macellaria*, and *L. eximia* was analysed (Godoy *et al*. 1996, 1997, 2001; Silva *et al*. 2003).

The introduced species *C. megacephala*, *C. putoria*, and *C. albiceps* showed a two-point limit cycle, whereas the native species *C. macellaria* and *L. eximia* exhibited a damping oscillation in population size leading to a fixed point equilibrium (Godoy *et al*. 1996, 1997, 2001; Silva *et al*. 2003). These results suggest that *L. eximia* and *C. macellaria* exhibit more stable dynamic behaviour than do *Chrysomya* species. Although these observations were obtained from experimental populations, the stability found makes sense, especially in view of the low seasonal variation found for natural populations of *L. eximia* (Gião and Godoy 2006).

L. eximia can apparently maintain a more stable population size than other calliphorid species, when facing environmental disturbances (Silva *et al*. 2003). This conjecture can be explained by the lack of seasonal variations or particular habitat preferences in this species (Moura *et al*. 1997). Linhares (1981) investigated the annual variation in the incidence of the calliphorid species in the Campinas region of the state of São Paulo, Brazil, and showed that *L. eximia* was relatively abundant all year round, exhibiting a much more stable population size than the *Chrysomya* species. We believe that the different responses to environmental disturbances produced by *L. eximia* may be, at least in part, associated with its plasticity. The genetic differentiation found in this study could explain its ability to maintain stable abundances through the seasons and at different geographic locations.

Biological invasions are extremely complex and difficult to interpret. Processes such as these can only be systematically evaluated over a long period of time (Hengeveld 1989). Intrinsic characteristics of invading species, including those genetic in nature, can determine the type of population response to the biological and physical influences of new environments (Groves and Burdon 1986). However, the gene analyses performed in this study revealed the existence of different haplotypes in three important blowfly species, *C. albiceps*, *C. macellaria* and *L. eximia*, which are involved in the biological invasion process.

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