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**Dinâmica e variabilidade populacional em dípteros
necrófagos: uma abordagem teórico-empírica**

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Dinâmica e variabilidade populacional em dípteros necrófagos:

uma abordagem teórico-empírica

Resumo Geral

A diversidade e abundância de Dípteros necrófagos foram investigadas em três áreas, urbana, rural e silvestre na cidade de Botucatu, Estado de São Paulo, Brasil, de março de 2003 a fevereiro de 2004, com objetivo de avaliar a distribuição e abundância de moscas no contexto forense. Espécimes da família Sarcophagidae foram os mais abundantes, seguidos por Drosophilidae, Calliphoridae e Phoridae. Espécimes de Muscidae foram os menos abundantes. As moscas foram mais abundantes na primavera e verão do que no outono e inverno. Espécimes de Sarcophagidae, Calliphoridae e Phoridae foram os mais abundantes na área urbana. *Chrysomya albiceps* foi a espécie mais abundante da família Calliphoridae, seguida por *Lucilia eximia*, *Chrysomya megacephala*, *Cochliomya macellaria* e *Lucilia cuprina*. Neste estudo também foram analisados dados de campo obtidos por censos populacionais de moscas varejeiras, obtidos durante dois anos em três diferentes áreas, urbana, rural e silvestre, com um modelo de dependência da densidade estruturado para a análise de três fragmentos, a fim de investigar a dinâmica populacional e persistência teórica de duas abundantes espécies de moscas varejeiras, a espécie exótica *C. albiceps* e a espécie nativa *L. eximia*. A análise da filogenia molecular também foi realizada com espécies de moscas varejeiras de importância forense, originárias de diferentes localidades. A análise genética revelou a existência de diferentes haplótipos em *Chrysomya albiceps*, *Cochliomyia macellaria*, e *Lucilia eximia* e mostrou através de três topologias a existência de linhagens mitocondriais bem definidas entre as moscas varejeiras exóticas e

nativas. Baseado na seqüência de dados foram formados sete *clusters* congênericos distintos. Os resultados foram discutidos em um contexto genético, ecológico e forense.

Abstract

The diversity and abundance of necrophagous Diptera were investigated in urban, farm and wild areas in Botucatu, São Paulo State, Brazil, from March 2003 through February 2004, in order to evaluate the current distribution and abundance of flies important in a forensic context. Members of the family Sarcophagidae were most abundant, followed by Drosophilidae, Calliphoridae and Phoridae. Members of Muscidae were least abundant. Flies were more abundant in spring and summer than in fall and winter. Members of Sarcophagidae, Calliphoridae and Phoridae were most abundant in urban areas. *Chrysomya albiceps* was the most abundant calliphorid species, followed by *Lucilia eximia*, *Chrysomya megacephala*, *Cochliomyia macellaria* and *Lucilia cuprina*. This study was also an attempt to connect field data obtained from blowfly populations censused for two years in three different areas, urban, farm and wild, with a simple density-dependent three-patch model, in order to investigate the theoretical population dynamics and persistence of two abundant blowfly species, the exotic *Chrysomya albiceps* and the native *Lucilia eximia*. A molecular phylogeny analysis was also performed on blowfly species of forensic importance from different localities. 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, ecological, and forensic contexts.

Introdução Geral

A trajetória temporal em populações de insetos é importante para a dinâmica das espécies e da comunidade na qual os organismos estão inseridos (Stiling, 1996). Entretanto, a trajetória populacional pode ser caracterizada por flutuações influenciadas por fatores endógenos, tais como parâmetros demográficos e exógenos, como, por exemplo, os fatores ambientais (Dennis *et al.*, 1995). Diversos fatores têm sido indicados como responsáveis por flutuações populacionais e a regulação populacional tem sido convencionalmente associada à dependência da densidade (Stiling, 1996). Em densidades suficientemente altas a mortalidade per capita excede a natalidade, levando a população ao declínio. Em baixas densidades o processo é reverso. Apesar da realidade implícita deste conceito, já que nenhuma população natural cresce ilimitadamente (Gotelli, 1995), outros fatores além da dependência da densidade podem também ser limitantes, fazendo parte dos processos reguladores naturais (Roughgarden, 1998).

A variação em valores demográficos que governam o crescimento populacional tem se mostrado um fator fundamental para o equilíbrio da população podendo resultar em transições no comportamento dinâmico, desde o equilíbrio estável, passando por oscilações periódicas até oscilações aperiódicas no tamanho populacional (Roughgarden, 1998). O significado desses resultados torna-se maior quando aplicado à populações biológicas, como demonstrado por Dennis *et al.* (1995) e Desharnais (2005), que observaram a transição de ciclos estáveis para o caos contínuo através da variação das taxas de natalidade

e mortalidade em populações de insetos. Essas variações no comportamento dinâmico parecem ter implicações para as taxas de extinção das espécies, já que a estabilidade populacional pode influenciar a probabilidade de persistência de populações (Desharnais, 2005).

A teoria da dinâmica populacional tem sido empregada para estudar cinco espécies de dípteros necrófagos da família Calliphoridae, *C. megacephala*, *C. putoria*, *C. albiceps*, *C. macellaria* e *L. eximia*, ao longo dos últimos quinze anos (Reis *et al.*, 1996; Godoy *et al.*, 1996, 1997, 2001; Silva *et al.* 2003; Castanho *et al.* 2006; Serra *et al.* 2006). Os estudos revelaram importantes diferenças na dinâmica de equilíbrio populacional entre as espécies introduzidas *C. megacephala*, *C. albiceps* e *C. putoria* e as espécies nativas *C. macellaria* e *L. eximia* (Reis *et al.* 1996; Godoy *et al.* 2001; Silva *et al.*, 2003).

Análises teóricas através de um modelo matemático que incorpora o processo de dependência de densidade e a estrutura espacial foram realizadas para investigar a dinâmica espaço-temporal de *C. megacephala*, *C. albiceps*, *C. putoria* e *C. macellaria* (Reis *et al.*, 1996; Godoy *et al.*, 1997; Godoy *et al.*, 2001). Os resultados indicam que as espécies introduzidas do gênero *Chrysomya* apresentam um ciclo limite estável de dois pontos, caracterizado pela oscilação entre dois valores representativos do tamanho populacional em função do tempo, um máximo e outro mínimo. As espécies nativas, *C. macellaria* e *L. eximia*, exibem um equilíbrio estável monotônico cujo significado biológico é a estabilização do tamanho populacional em um único valor (Godoy *et al.*, 1996; Reis *et al.*, 1996; Godoy *et al.*, 2001; Silva *et al.* 2003). Estes resultados são importantes no contexto da dinâmica populacional, posto que uma nítida diferença no comportamento dinâmico entre as espécies introduzidas e as espécies nativas foi constatada.

A hipótese do deslocamento das espécies nativas pelas espécies introduzidas, tem sido testada através de experimentos delineados para analisar interações interespecíficas entre espécies do gênero *Chrysomya* e *C. macellaria* (Faria *et al.* 1999; Rosa *et al.* 2006). Os resultados desses estudos revelam que as espécies do gênero *Chrysomya* tem melhor habilidade competitiva que *C. macellaria* e que a predação intraguilda exibida por *C. albiceps* tem provavelmente contribuído com o declínio do tamanho populacional de *C. macellaria* (Faria *et al.*, 1999; Rosa *et al.* 2006).

A finalidade deste estudo foi obter dados em campo na tentativa de refinar as estimativas de parâmetros demográficos, necessários à descrição de padrões de comportamento dinâmico, em modelos populacionais aplicados ao crescimento populacional, de forma que as proposições teóricas pudessem estar fundamentadas em estimativas de populações naturais. O presente estudo deu ênfase à coleta quinzenal de dípteros necrófagos em três áreas (urbana, rural e silvestre) município de Botucatu, São Paulo durante o período de dois anos. Entretanto, com o monitoramento dos resultados optou-se pela ampliação da análise populacional, estendendo-a, principalmente com vistas à investigação molecular, a outros municípios, incluindo outros estados brasileiros. As pesquisas produziram resultados interessantes que deram origem a três publicações científicas, as quais integram os capítulos desta tese.

O primeiro artigo analisa a abundância sazonal e distribuição dos espécimes coletados, incluindo as seguintes famílias: Sarcophagidae, Calliphoridae, Drosophilidae, Phoridae e Muscidae. No segundo trabalho foi feito um estudo da dinâmica metapopulacional com acoplagem de modelos matemáticos clássicos da literatura para analisar, sob a perspectiva espaço-temporal, a dinâmica de *Lucilia eximia* e *Chrysomya albiceps*, as duas espécies de califorídeos mais abundantes no decorrer do estudo. O último

artigo, consiste de uma análise molecular com emprego de DNA mitocondrial, para espécies coletadas nos municípios de Botucatu-SP, Gramado-RS, Presidente Prudente-SP e Nova Andradina-MS. Acreditamos que a abordagem proposta tenha gerado resultados interessantes e importantes, contribuindo assim com a constituição do banco de dados em Entomologia Forense no Brasil.

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Seasonal abundance and distribution of necrophagous Diptera in western São Paulo State, Brazil

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Abstract: The diversity and abundance of necrophagous Diptera were investigated in urban, farm and wild areas in Botucatu, São Paulo State, Brazil, from March 2003 through February 2004, in order to evaluate the current distribution and abundance of flies important in a forensic context. Members of the family Sarcophagidae were most abundant, followed by Drosophilidae, Calliphoridae and Phoridae. Members of Muscidae were least abundant. Flies were more abundant in spring and summer than in fall and winter. Members of Sarcophagidae, Calliphoridae and Phoridae were most abundant in urban areas.

Chrysomya albiceps was the most abundant calliphorid species, followed by *Lucilia eximia*, *Chrysomya megacephala*, *Cochliomyia macellaria* and *Lucilia cuprina*. The implications of these results for the necrophagous fauna structure and forensic investigations are discussed.

Keywords: Seasonal abundance, necrophagous flies, forensic entomology.

1 Introduction

Forensic entomology has in recent years become an increasingly important part of the forensic sciences (Wolff et al., 2001; Catts and Haskell, 1997). It has been applied mainly to estimate the time of death or postmortem interval (PMI), based on the developmental rates and the successional ecology of specific insects that feed on carcasses (Anderson, 2001).

After death, animal tissues, including those of humans, are attractive to many kinds of organisms, especially insects. Hence, the decomposition of terrestrial vertebrates is characterised not only by the action of fungi and bacteria, but also by an ample number of arthropods, mainly necrophagous insects (Anderson, 1995; Amendt et al., 2004). In addition, the kind of death markedly affects decomposition, because it determines how fast a corpse can reach putrefaction (Anderson, 1995). Insects and other invertebrates feed on carrion in a succession that is dependent upon the state of decomposition. Recognition of the species involved, the pattern and time of arrival at the scene of the adults, and subsequently the eggs and larvae, together with knowledge of their development rates, can give an indication of the time of death (Anderson, 1995).

Season has an important impact on the weather and the flora and fauna of a region, which influence significantly the faunal colonisation of a body. Many fly species vary in abundance depending upon season. For example, in Mississippi, *Lucilia coeruleiviridis* and *Cochliomyia macellaria* were dominant in the warmer summer months, whereas *Calliphora livida* and *Cynomyopsis cadaverina* dominated in the winter months, with *Phormia regina* found throughout the year (Goddard and Lago, 1985).

Studies of succession and decomposition in carcasses have been done mostly in temperate countries (Arnaldos et al., 2004). However, research programs have also been implemented in Brazil, in an attempt to understand the dynamics of these insects in tropical areas and their association with forensic studies (Von Zuben et al., 1996; Carvalho et al., 2000, 2004).

Population abundance in necrophagous flies has usually been estimated from periodic census by using traps (Martinez-Sanchez et al., 2000). The abundance of these species has been investigated by field succession experiments, which involved capture of flies from pigs in specific areas (Souza and Linhares, 1997).

The relative abundance of certain insects and the potentially differing time of colonisation of the remains in different seasons are essential factors to understand the succession process in corpses (Smith, 1986). Studies of this nature should be performed throughout the year, in order to develop a valid database for specific areas. Insects may be valuable in determining season of death, and the database can be useful when remains are discovered several years after death (Anderson, 1995; Catts and Haskell, 1997). In Brazil, no systematic study has investigated the abundance of necrophagous flies in different places, such as urban, wild and farm areas, especially in locations in which the environmental conditions differ in terms of altitude and temperature.

In this study, we investigated the diversity and abundance of necrophagous Diptera in Botucatu, São Paulo State, Brazil, from March 2003 through February 2004, in order to evaluate the current distribution of species. This area includes urban, wild and farm areas. We believe that this information can significantly increase the level of knowledge of fly diversity associated with carcasses, and consequently provides important information on flies as forensic indicators.

2. Material and methods

Bimonthly collections were made during the course of one year, March 2003 through February 2004, in three different areas: urban, farm and wild. The urban traps were set in a town garden, near a residential area. The farm traps were set in the Experimental Farm of São Paulo State University, Botucatu, São Paulo, near the university campus. The wild area was a semideciduous forest near the farm.

Traps were made from plastic drinking bottles (2000 mL), each with a hole in its bottom (9 cm diameter X 30 cm length). Chicken viscera, were placed in the bottles as bait. Six traps were set in trees: three in the shade and three in the sun. Because the number of specimens found in the shade was not significantly different from individuals found in the sun ($p > 0.05$), all data were pooled for analysis. The traps were removed after 72 h and the flies identified and recorded. Except for members of the family Calliphoridae, the flies were identified only to family level.

Members of Calliphoridae were identified to species because of the need to record the current status of the group in terms of abundance and distribution of native and exotic species. The structure of the Brazilian calliphorid fauna has changed since the biological invasion of species of *Chrysomya* about 30 years ago (Guimarães et al., 1978).

One-way ANOVA was employed to compare the difference in terms of abundance among areas, families and species, in the case of Calliphoridae. Mean monthly temperatures for the Botucatu area were obtained from the Meteorological Station of São Paulo State University in Botucatu, which is located near the three experimental areas. All traps were placed at a distance of 3 kilometres from the Meteorological Station.

The frequency distribution of flies in traps was fitted to the Negative binomial and Poisson distributions, in order to determine whether the number of adults found among traps was clumped or random. The k parameter in the Negative binomial distribution was estimated by the maximum likelihood method (Ludwig and Reynolds, 1988). The fits of the Negative binomial and Poisson distributions were tested by the Pearson χ^2 statistic (Ludwig and Reynolds, 1988). In the Negative binomial distribution, the null hypothesis was that the frequency distribution of adults exhibited a clumped distribution pattern. Parameter k is a measure of the degree of clumping, and tends toward zero at maximum clumping. In the Poisson distribution, the null hypothesis was that the number of adults found follows a random distribution.

3. Results and discussion

The collections resulted in 1,503 specimens, members of five families: Sarcophagidae, Calliphoridae, Drosophilidae, Phoridae and Muscidae (Table 1). Members of Sarcophagidae were most abundant, with 590 specimens. There were 533 individuals of Drosophilidae, 227 of Calliphoridae and 140 of Phoridae. Only 13 individuals of Muscidae were collected over the period (Table 1).

The Sarcophagidae, commonly called flesh-flies, is a large family, with over 2000 species of cosmopolitan distribution (Smith, 1986). Sarcophagids occur in tropical and warm-temperature regions, with adults observed often on flowers, feeding on sweet substances, including sap and honeydew (Smith, 1986). In addition to carrion, they also may feed on excrement or exposed meat (Smith, 1986; Wolff et al., 2001). Flesh-flies are attracted to carrion under most conditions, including sun, shade, dry, wet indoors, and outdoors (Wolff et al., 2001). Some sarcophagids have evolved into parasitoids, attacking live insects, with

Orthoptera as particularly common hosts; other species live in nests of hymenopterans and termites, eating the food stored for the original insect larva, and often the larva itself (Ferrar, 1987).

Members of the family Drosophilidae were the second most abundant group collected. Species of Drosophilidae are attracted to practically any fermenting substance, with more than 2000 known species, widely distributed by commercial traffic (Smith, 1986).

Drosophilids are commonly found in breweries, public houses, pickling factories, fruit and vegetable canneries, canteens and restaurants; some species are found on carrion, principally when putrid liquids exude (Atkinson, 1985; Smith, 1986). High variability in terms of relative abundance seems common for some species of fruit flies (Beaver, 1977; Atkinson, 1985).

We can suggest no clear reason for the higher abundance of Sarcophagidae and Drosophilidae than Calliphoridae found during this study. Most time-series studies of necrophagous Diptera suggest that the calliphorids are the most abundant family of flies captured (Carvalho et al., 2000; Carvalho and Linhares, 2001; Carvalho et al., 2004). One question arising from these results, is whether the trap design can influence the abundance and diversity of flies captured. Several trap designs are employed in studies of this nature (Hall, 1995). However, the trap used in our investigation is very similar to the trap employed by Hwang and Turner (2005), who developed a bottle trap made from soft plastic drinking bottles. They observed that the Calliphoridae was the most abundant family captured in the London area (Hwang and Turner, 2005).

Another reason for our result may be the degree of humidity of the bait, chicken viscera. Certainly the age of the bait and its stage of decomposition are also important factors capable of affecting the number, sex and age composition of blowfly populations (Vogt and

Woodburn, 1994). During the period of time when the traps were maintained in the field, we observed that the bait was dried by the wind, which in this area has a mean velocity of 14 km/h. This drying may have contributed to make the bait less attractive to calliphorids, which are usually the first species to arrive in carcasses (Smith, 1986). In addition, the local altitude is 840 m, making possible differences in terms of abundance and diversity of flies compared to lower areas (Mani, 1968). As altitude increases, conditions for life become more rigorous, with food becoming scarce, humidity and temperature falling, and the temperature oscillating much more (Mani, 1968).

Calliphoridae, a family with over 1000 described species that are widely distributed in all zoogeographical regions (Smith, 1986), was the third most abundant taxonomic group. Of all the calliphorids collected, the highest abundance was recorded for *C. albiceps*, with 136 specimens, followed by *L. eximia* with 80 specimens, *C. megacephala* with 20 specimens, *C. macellaria* with 8 specimens and *L. cuprina* with only 1 individual (Table 2). However, *C. albiceps* was not recorded during four months, whereas *L. eximia* was observed all year (Table 2). The highest abundance of *C. albiceps*, *L. eximia* and *C. megacephala* was recorded in the urban area. The farm area was where *C. macellaria* was the most abundant (Table 2). The abundance among calliphorid species was significantly different only in the wild area (Table 2).

The structure of the Brazilian necrophagous fauna, particularly Calliphoridae, has been influenced by the abundance of exotic blowflies such as species of *Chrysomya*, which were introduced into the Americas about 30 years ago (Guimarães et al., 1978). Four species of *Chrysomya* have been introduced into the New World (Guimarães et al., 1978): *Chrysomya megacephala* (F.), *C. putoria* (Wiedemann), *C. albiceps* (Wiedemann) and *C. rufifacies* (Wiedemann). These species originally occurred in Australia, the Oriental Region, and

Africa, and were first detected in South America around 1975, except *C. rufifacies* which has been found only in North America (Guimarães et al., 1978). The successful biological invasion, colonisation and persistence of *Chrysomya* species in different regions of the world can be explained by their short life cycle and high growth rate (Smith, 1986; Godoy et al., 1993). Particularly in tropical areas such as Brazil, introduced blowflies found a suitable environment to maintain their populations at high levels (Guimarães et al., 1978; Smith, 1986; Souza and Linhares, 1997)

Of the Calliphoridae, *C. albiceps* was the most abundant species in the urban and farm areas. This may be attributed to its predatory habit on other species and its rapid development (Faria et al., 1999). The conspicuous abundance of *C. albiceps* was also observed in urban areas in Campinas, São Paulo, Brazil; Rio de Janeiro; Goiás; and Curitiba, Paraná, Brazil (Moura et al., 1997; Souza et al., 1997; Carvalho et al., 2004).

In spite of its lower abundance compared to *C. albiceps*, *L. eximia* was collected during all seasons, differing in this regard from *C. albiceps*. *Lucilia eximia* is able to maintain high abundances in both urban and wild areas during all seasons (Moura et al., 1997), which could explain the persistence of this species over the course of the year. The least abundant species were *L. cuprina* and *C. macellaria*. The low abundance of *C. macellaria* is easily explained, because it has been strongly influenced by the invasion of *Chrysomya* species about 30 years ago (Guimarães et al., 1978; Faria et al., 1999).

Most species of Phoridae were collected in the urban and wild areas (Table 1). This is a large family of flies, with some 3000 species (Smith, 1986). Phorids breed in a wide variety of decaying organic material, and several genera are regularly found in vertebrate carrion (Smith, 1986). The variety of substrates utilised by the species explains their presence in the traps, but we can suggest no specific reason to find them more abundantly in the urban

and wild areas than in the farm area. The family Muscidae exhibited the lowest abundance. This result was not expected, because muscids have often been abundant in studies performed in different areas (Smith, 1986; Axtell and Arends, 1990). We believe that the principal reason for this result is the presence of a poultry house near the farm area, which may have attracted the flies to the high concentration of chicken excrement, compared to the bait used in our investigation.

The urban area was where the highest abundance was recorded for Calliphoridae, but also for Sarcophagidae and Phoridae (Table 1). Drosophilidae and Muscidae were most abundant in the farm area (Table 1). The difference in terms of abundance of flies was significant among families in the urban ($p < 0.05$) and wild areas ($p < 0.05$). Excluding Muscidae from the wild-area comparison, the same result was found. However, in the farm area, no significant difference was found in terms of abundance of flies ($p > 0.05$).

No significant correlation was found between temperature and abundance of flies, and rainfall and abundance. However, during summer and spring, flies were more abundant than during fall and winter. The absence of a significant correlation between weather conditions and abundance has also been observed in other geographic areas, for example Malaysia. A study in Malaysia showed that the number of specimens of *C. bezziana* caught was unaffected by weather conditions at the time of trapping, but was positively correlated with the total rainfall (Mahon et al., 2004).

The frequency-distribution analysis of adults revealed that the clumped pattern of distribution, described by the Binomial negative model, was the most prevalent pattern of distribution (Table 3). A few areas within families showed a random pattern characterised by the Poisson model (Tables 3 and 4), probably as a function of the low abundance recorded. Calliphoridae exhibited the closest value to zero for the k parameter among the

families, indicating the highest degree of clumping. This pattern of distribution was observed because blowflies usually tend to search for substrates previously visited by other individuals of the same family. Adult aggregation in blowflies has been frequently documented (Cruickshank and Wall, 2002), and this behaviour has been understood as a strategy to increase egg crowding, promoting proteolytic enzyme production by larvae after they hatch (Smith, 1986).

The approach taken here to analyse frequency distribution of flies has been often employed in studies to search for spatial patterns in the distribution of invertebrates, particularly parasites and insects (Sréter et al., 1994; Reigada and Godoy, 2005). Most of these studies have investigated the effect of distribution patterns of eggs and larvae among discrete patches on the coexistence of competing species. These analyses have also been used to investigate aggregated patterns as a consequence of post-feeding larval dispersal in three blowfly species, *C. macellaria*, *C. megacephala* and *C. putoria*, and recently to analyse the influence of larval predation on the dispersal of blowfly larvae (Reigada and Godoy, 2005).

The abundance recorded for flies over the study period confirms the results obtained in several fly censuses in Brazil (Carvalho et al., 2000, 2004). These studies also showed that members of Calliphoridae and Sarcophagidae were most abundant. Summer and spring were the seasons in which the highest abundance of flies was observed, as noted in several studies (Carvalho et al., 2000; Centeno et al., 2002).

Comparing the results found in this study with data from other regions such as Argentina, the United States, Australia, New Zealand, the Iberian Peninsula, Spain, Austria, Egypt and India, important differences in terms of diversity and abundance can be observed. The

seasonal pattern of arthropods in Buenos Aires was favorable to the presence of *Calliphora vicina*, but *C. macellaria* and *Lucilia cluvia* were also recorded (Centeno et al., 2002).

A large-scale study of the patterns of neonatal piglet decomposition and carrion insect succession carried out in southern Victoria, Australia, revealed that *Calliphora augur*, *Chrysomya rufifacies* and *C. varipes* were the calliphorid species most abundant in 1999 and 2000, except during June and July (Archer and Elgar, 2003). *Lucilia sericata* was the most commonly trapped calliphorid species in the South Island of New Zealand, followed by *Calliphora hilli*, *C. stygia*, *C. vicina*, *C. quadrimaculata*, *Chrysomya rufifacies* and *Xenocalliphora hortona* (Barrat et al., 2001).

A study of the sarcosaprophagous community in the southeastern Iberian Peninsula during the four seasons, evaluated different decomposition stages, fresh, decomposing and advanced decomposition. The investigation revealed that *L. sericata* was the most abundant species of calliphorid in all decomposition stages and seasons, followed by *C. vicina*, *C. albiceps*, *Pollenia* sp. and *C. vomitoria* (Arnaldos et al., 2004).

In Central Europe, *C. vomitoria* and *C. albiceps* have been found in abundance, with larvae and adults of *C. vomitoria* outnumbering all other blowfly species, followed by *Protophormia terraenovae*, *C. vicina* and *L. sericata* (Grassberger and Frank, 2004). *Chrysomya albiceps* has been found in Austria, monopolising carcasses probably as a consequence of its predatory behaviour during the larval stage (Verves, 2004; Grassberger and Frank, 2004). *Lucilia sericata* and *C. albiceps* were the principal species coexisting in carrion in fall and spring in Egypt (Adham et al., 2001).

Comparing the results found in this study with previous investigations in Brazil and other geographic locations, it is possible to conclude that *C. albiceps* is consistently abundant.

This is certainly associated with its predatory habit, experimentally confirmed (Faria et al., 1999).

However, in some areas in the Northern Hemisphere, the genera *Lucilia* and *Calliphora* apparently dominate the fauna, even when *C. albiceps* is present (Grassberger and Frank, 2004). Differences in terms of ovipositional succession in response to carcass decomposition stage and temperature could explain the success of *Lucilia* and *Calliphora* in spite of the presence of *C. albiceps* (Grassberger and Frank, 2004). The absence of *Calliphora* from our traps confirms that this species is not present in the western part of São Paulo State, as observed in previous studies (Moura et al., 1997; Souza et al., 1997; Carvalho et al., 2000), although it is present in the southern part of the country (Carvalho and Ribeiro, 2000).

Abundance and distribution of necrophagous Diptera are essential factors to be considered in forensic studies, since the diversity and numbers of flies can improve comprehension of the fauna associated with the decomposition of corpses, and consequently clarify questions concerning criminal acts (Grassberger and Frank, 2004).

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Table 1: Abundance and distribution of individual flies, classified by family and area

Month	Calliphoridae			Sarcophagidae			Drosophilidae			Phoridae			Muscidae			Σ
	Urban	Farm	Wild	Urban	Farm	Wild	Urban	Farm	Wild	Urban	Farm	Wild	Urban	Farm	Wild	
Mar	18	1	1	38	16	5	65	346	10	0	3	28	1	2	0	534
Apr	19	0	2	49	11	13	0	0	0	0	0	0	0	0	0	94
May	7	0	1	11	12	6	0	0	0	0	0	0	0	0	1	38
Jun	21	0	2	48	15	15	1	0	0	0	0	0	0	0	0	102
Jul	0	2	0	6	1	1	0	0	2	13	0	19	0	0	0	44
Aug	0	0	1	1	4	20	1	2	18	0	0	1	0	1	0	49
Sep	1	0	0	2	7	20	0	0	1	3	0	10	0	0	0	44
Oct	3	0	1	21	14	0	0	0	1	0	0	1	0	5	2	48
Nov	106	21	1	104	30	42	47	7	6	24	4	1	0	0	0	393
Dec	12	0	1	29	5	16	8	7	0	16	1	0	0	0	0	95
Jan	3	0	1	8	4	13	0	3	7	6	6	0	1	0	0	52
Feb	2	0	0	3	0	0	0	1	0	2	2	0	0	0	0	10
Σ	192	24	11	320	119	151	122	366	45	64	16	60	2	8	3	1503

Table 2: Abundance and distribution of calliphorid flies, by species and area.

Month	<i>C. albiceps</i>			<i>L. eximia</i>			<i>L. cuprina</i>			<i>C. megacephala</i>			<i>C. macellaria</i>			Σ
	Urban	Farm	Wild	Urban	Farm	Wild	Urban	Farm	Wild	Urban	Farm	Wild	Urban	Farm	Wild	
Mar	10	1	0	9	0	1	0	0	0	0	0	0	1	0	0	22
Apr	3	0	0	16	0	2	0	0	0	0	0	0	0	0	0	21
May	5	0	0	2	0	0	0	0	0	0	0	1	0	0	0	8
Jun	7	0	0	13	0	1	1	0	0	0	0	1	0	0	0	23
Jul	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2
Aug	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
Sep	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Oct	1	0	0	2	0	1	0	0	0	0	0	0	0	0	0	4
Nov	86	13	3	6	3	1	0	0	0	13	0	1	1	5	1	133
Dec	4	0	1	8	0	0	0	0	0	3	0	0	0	0	0	16
Jan	2	0	0	8	0	1	0	0	0	1	0	0	0	0	0	12
Feb	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2
Σ	118	14	4	67	5	8	1	0	0	17	0	3	2	5	1	245

Table 3: Frequency distribution of flies among traps, by family and area

		Mean	s ²	K	X ²	df	Test
Calliphoridae	Urban	8.916	75.35	0.06752	14.04	17	**
	Farm	2	36.18	0.0921	2.4423	11	**
	Wild	0.916	0.446		3.000471	2	*
Sarcophagidae	Urban	21.9166	373.57		1.51934	48	*
	Farm	9.91	69.9	1.065	31.55	29	**
	Wild	12.58	140.447	0.5712	50.6855	36	**
Drosophilidae	Urban	10.166	478.15	0.1214	9.9897	19	**
	Farm	2.25	9.11	0.3425	1.527	3	**
	Wild	3.75	31.47	0.3592	24.4788	17	**
Phoridae	Urban	5.333	64.42	0.2125	23.3942	21	**
	Farm	1.333	4.0606	0.3365	5.42814	5	**
	Wild	5	86.1818	0.2184	24.72556	20	**
Muscidae	Urban	0.166	0.1515		0.20739	1	*
	Farm	0.666	2.2424	0.192	6.3952	4	**
	Wild	0.25	0.38	0.303	1.208	1	**

*Poisson distribution (P<0.001)

**Negative binomial (P<0.05)

Table 4: Frequency distribution of flies among traps, by species and area

Calliphoridae		Mean	s ²	K	χ ²	df	Test
<i>C. albiceps</i>	Urban	9.83	585.42	0.31	12.46	64	**
	Farm	1.16	13.96	0.06	2.03	8	**
	Wild	0.333	0.78	0.165	3.72	2	**
<i>L. eximia</i>	Urban	5.58	28.08	0.913	22.59	15	**
	Farm	0.416	0.99	0.1235	4.85	2	**
	Wild	0.666	0.4242		1.5529	2	*
<i>C. megacephala</i>	Urban	1.416	14.08	0.109	3.85	10	**
	Wild	0.25	0.2045		0.519	1	*
<i>C. macellaria</i>	Urban	0.1666	0.1515		0.207	1	*
	Farm	0.416	2.08	0.035	1.54	2	**

*Poisson distribution (P<0.001)

**Negative binomial (P<0.05)

Metapopulation dynamics of blowflies: a three-patch system combining empiricism and theory

Abstract The spatial component of population dynamics has inspired a variety of mathematical formalisms, and several types of models have been used to explore the role of metapopulation and community dynamics. This study is an attempt to connect field data obtained from blowfly populations censused for two years in three different areas, urban, farm and wild, with a simple density-dependent three-patch model, in order to investigate the theoretical population dynamics and persistence of two abundant blowfly species, the exotic *Chrysomya albiceps* and the native *Lucilia eximia*. Specifically, the study had the objective to investigate theoretical temporal trajectories of blowflies, considering random migration between predetermined boundaries among different patches. Stochasticity was also applied to carrying capacity and growth rate. The results, after analysis by the three-patch deterministic model, suggest a stable equilibrium for both species. The stochastic analysis showed that the variation in carrying capacity between predetermined boundaries without migration leads the populations to global extinction within a few generations. The same result was not obtained when stochastic migration was incorporated. The stochastic growth rate produced local persistence, and the addition of migration produced global persistence. The simultaneous stochastic carrying capacity and growth rate led to global extinction within a few generations, but the addition of migration resulted in an increase of persistence, and, for some populations, also produced spatial synchrony.

Keywords Metapopulation • stochasticity • blowflies • population theory

Introduction

Metapopulation theory has been applied to investigate several biological systems, considering different aspects, principally in conservation biology (Bascompte 2001; Alonso and Mckane 2002; Casagrandi and Gatto 2002; Grez et al. 2004). However, the complexity of many

species can make it difficult to analyse natural population dynamics (Desharnais 2005). In such situations, the best solution may be the implementation of theoretical studies combined with field data sets (Hanski 1999). The metapopulation approach has been empirically and theoretically applied to laboratory populations in order to evaluate essential aspects of migration in different organisms (Hanski and Gilpin 1997).

An understanding of the processes leading to population fluctuations in a metapopulation context with environmental heterogeneity, as well as persistence and/or extinction, is important for many questions in population biology, such as life history evolution, the success of colonising species, and the management of endangered species and zoo populations (Hanski 1999). The causes of extinction may be related to several factors: demographic processes, such as random fluctuations in birth and death rates and sex ratio; seasonal and other changes in the environment, including predation and competition; catastrophes; disease outbreaks; and genetic problems, including the accumulation of deleterious mutations or the loss of adaptive variation (Lawton and May 1995).

The effect of random environmental variation on population dynamics has also been well documented (Goodman 1987; Pimm 1991; Ariño and Pimm 1995). A population in a variable environment with exchange of individuals between subpopulations will experience variation in both time and space. At any given moment, each subpopulation may not be perfectly correlated with other subpopulations (Ranta et al. 1995). Hence, both the degree of correlation with environmental variation and the dispersal pattern among subpopulations could affect both local and global dynamics.

Nevertheless, in spite of the important role that migration plays in preventing local or global extinctions or re-colonisation of habitats, some random biological events may affect the persistence of populations even when they are strongly connected with spatial migration (Ovaskainen et al. 2002). Demographic and environmental stochasticity can strongly influence both local population dynamics and the synchrony between them (Gotelli 1995).

The spatial component of population dynamics has inspired a variety of mathematical formalisms, which differ in detail (Hanski 1994). Several types of models have been used to explore

the role of metapopulation and community dynamics (Taylor 1988; Kareiva 1990; Hanski 1991, 1994). Some metapopulation models are based on measures of presence or absence in habitat patches interconnected by migration (Hanski 1991, 1994). They are stochastic because colonisation and extinction of patches are random events contingent on patch area and relative spatial isolation (Roughgarden 1998; Renshaw 1999).

Blowflies can produce myiasis in humans and other animals, and can also transmit pathogens mechanically (Baumgartner and Greenberg 1984; Guimarães and Papavero 1999). Also, interest in these flies has grown because they can serve as biological indicators of the time of death in forensic medicine (Amendt et al. 2004). The exotic species *Chrysomya megacephala*, *C. putoria* and *Chrysomya albiceps*, common and abundant in the tropics and subtropics of the Old World and Oceania, were introduced and first detected in South America around 1975 (Guimarães et al. 1978) and have since become established in the Americas (Baumgartner and Greenberg 1984).

The invasion of these species has apparently caused a negative impact on the population numbers of two native species, *Cochliomyia macellaria* and *Lucilia eximia*. *Cochliomyia macellaria* has been strongly influenced by the exotic species, whereas the impact on *Lucilia eximia* has been less pronounced (Guimarães et al. 1979; Madeira et al. 1989). This invasion displays some of the classical outcomes of similar events in other areas; that is, the rapid spread of invaders and the concomitant decline of native species at the local and macrogeographic scales (Lodge 1993).

The invasion of new habitats by organisms is an important ecological phenomenon, because invading species generally have tremendous ecological and economic impact on new areas (Hengeveld 1989; Kareiva 1996). Biological invasions can take place in different ways, including invasions into patchy environments and by stratified diffusion both in short- and long-range dispersal (Shigesada and Kawasaki 1997). The consequences of an invasion may vary from competition for food or space between invading and native species, to invasion of parasites and the spread of epidemic diseases (Hengeveld 1989; Shigesada and Kawasaki 1997).

Invader populations depend upon physical and biological factors for success in the invasion and colonization process (Stiling 1996). Among the main biological factors associated with population

growth as well as success in colonisation, persistence and extinction of populations in new areas deserve special attention because the future of an invading species in its new habitat depends basically upon the period of time that it remains in the new habitat (Hengeveld 1989; Caughley and Gunn 1996; Hanski 1999).

The biological invasion of blowflies into South America has afforded a profitable scenario to investigate the population dynamics of the introduced species of the genus *Chrysomya* and of the native species *C. macellaria* and *L. eximia* (Godoy et al. 2001; Silva et al. 2003). Recently, the population dynamics of introduced and native species was investigated in a metapopulation context by using Fuzzy subset and stochastic simulations, in an attempt to understand how environmental and demographic influences can affect the population dynamics of blowflies (Castanho et al. 2006; Serra et al. 2006).

Although these studies have improved comprehension of the dynamics and persistence of exotic and native blowflies, the data set used to analyse the system was obtained in the laboratory during a long period of experimental work (Godoy et al. 1996, 1997; Reis et al. 1996; Silva et al. 2003; Godoy et al. 2001; Castanho et al. 2006; Serra et al. 2006). There is no field data set available for exotic and native species in Brazil, which can be analysed as a time series, combining empiricism and population theory in a metapopulation context.

This study is an attempt to connect field data obtained from blowfly populations censused for two years in three different areas, urban, farm and wild, very close to each other, with a simple density-dependent three-patch model, in order to investigate the theoretical population dynamics and persistence of two abundant blowfly species, *C. albiceps* and *L. eximia*, an exotic and a native species respectively. The study had the specific objective to investigate theoretical temporal trajectories of blowflies, considering random migration between predetermined boundaries among different patches, with stochasticity applied to carrying capacity and population growth.

Materials and methods

Collections were made twice monthly for two years, March 2003 through February 2005, in three different areas: urban, farm and wild. The urban traps were set in a town garden, near a residential area. The farm traps were set on the Experimental Farm of São Paulo State University, Botucatu, São Paulo, Brazil, near the university campus. The wild area consists of a semideciduous forest, near the farm area.

Traps were set from plastic drinking bottles (2000 mL), all of them with a hole on the bottom (9 cm diameter X 30 cm length). Chicken viscera, used as bait, were placed into the bottles. Six traps were set on trees, three in the shade and three in the sun. Because the number of specimens found in the shade was not significantly different from the number found in the sun ($p > 0.05$), all data were pooled for analysis. The traps were removed after 72 h and the flies identified and recorded. Individuals of *C. albiceps* and *L. eximia* were chosen to be analysed in this study because they are involved in the biological invasion process of *Chrysomya* species, which began about 30 years ago (Guimarães et al. 1978). All traps were placed at a distance of 3 kilometres from the Meteorological Station. The estimates obtained (Table 1) suggest that the two years were characterised by different conditions of population growth in response to different abundances. This scenario illustrates the concept of good and bad years that is usually employed in studies involving environmental stochasticity (Roughgarden 1998). The time series illustrated herein were produced by simulating the population dynamics of each species, based on carrying capacity and growth rate estimated from the census (Table 1).

Mathematical models

Metapopulation model: two-patch formalism

The model for two-patch populations can be written as

$$\begin{aligned}
n_{1,t+1} &= r_{1,t} [(1 - m_1)n_{1,t} + m_2 n_{2,t}] \\
n_{2,t+1} &= r_{2,t} [m_1 n_{1,t} + (1 - m_2)n_{2,t}]
\end{aligned} \quad . \quad (1)$$

In this model, m is the probability that an organism from patch 1 disperses to patch 2, and vice versa, i.e., it is the probability that an organism will migrate (Roughgarden 1998). Therefore, $(1-m)$ is the probability that an organism will remain in its original patch and will not migrate to another patch. $N_{x,t}$ is the number of individuals in the population at time t and location x , where x is 1 or 2. The geometric growth rate at location x at time t is r . If m is zero, the equations describe two separate uncoupled populations, and if m is $\frac{1}{2}$ the two populations are completely mixed and are in effect one population.

Ricker model

The simple discrete-time population model developed by Ricker (1952) has the desirable property that population size cannot become negative. In addition, as other models, it has played an important role in the description of non-linear dynamics, an important characteristic for populations of insects, especially blowflies (Dennis et al. 1995; Godoy et al. 2001). The Ricker equation is generally written as

$$n_{t+1} = n_t e^{[r(1-\frac{n_t}{K})]} \quad (2)$$

where r and K set the growth rate and the carrying capacity, respectively. Combining the patch model with the Ricker formulation results in three equations, which describe the dynamics of three coupled populations as

$$\begin{aligned}
n_{1,t+1} &= (1 - m_{12})n_{1,t} e^{r_1(1-\frac{n_{1,t}}{K_1})} + (1 - m_{13})n_{1,t} e^{r_1(1-\frac{n_{1,t}}{K_1})} + m_{21}n_{2,t} e^{r_2(1-\frac{n_{2,t}}{K_2})} + m_{31}n_{3,t} e^{r_3(1-\frac{n_{3,t}}{K_3})} \\
n_{2,t+1} &= (1 - m_{21})n_{2,t} e^{r_2(1-\frac{n_{2,t}}{K_2})} + (1 - m_{23})n_{2,t} e^{r_2(1-\frac{n_{2,t}}{K_2})} + m_{12}n_{1,t} e^{r_1(1-\frac{n_{1,t}}{K_1})} + m_{32}n_{3,t} e^{r_3(1-\frac{n_{3,t}}{K_3})} \\
n_{3,t+1} &= (1 - m_{31})n_{3,t} e^{r_3(1-\frac{n_{3,t}}{K_3})} + (1 - m_{23})n_{3,t} e^{r_3(1-\frac{n_{3,t}}{K_3})} + m_{13}n_{1,t} e^{r_1(1-\frac{n_{1,t}}{K_1})} + m_{23}n_{2,t} e^{r_2(1-\frac{n_{2,t}}{K_2})}
\end{aligned} \quad (3),$$

where m is the migration rate between areas, 1 (urban), 2 (farm) and 3 (wild). Then, for example, m_{12} describes the migration from urban to farm area, m_{13} the migration from urban to wild area, and so on. Two growth rates (r_{year1} and r_{year2}) were employed in the simulations, obtained from the two annual censuses (Table 1) to simulate the effect of variation between two rates. Three growth rates for year 1 and year 2 (r_1 , r_2 , and r_3) were employed in the simulations obtained from *Urban (1)*, *Farm (2)* and *Wild (3)* areas, computed from the successive population sizes and transferred to the model as the geometric mean of the growth rates among the months in which the blowfly species were collected, since for biological populations in nature, one does use the geometric mean when grading a population success (Roughgarden 1998). The maximum numbers of each blowfly species captured in each area were used to express the carrying capacities.

The parameters K and r were allowed to fluctuate between the maximum and minimum values estimated (Table 2). The migration rate (m) was allowed to fluctuate between 0.4 and 0.6. These limits for migration were chosen in order to investigate the effects of high migration rates in a stochastic context on the population dynamics of introduced and native blowflies. In addition, they were the most suitable to show susceptibility to spatial synchrony between local populations in a previous study focused on persistence dynamics of blowflies (Serra et al. 2006). The function “rand” (Matlab 7.0.1) was used to simulate the stochastic dynamics with uniform distribution, in order to ensure that all of the values between the established boundaries had the same chance of occurrence. For each species and stochastic parameter, 1000 simulations were run using Matlab 7.0.1 (Hanselman & Littlefield 1997).

Statistical analysis

The spatial synchrony was analysed by comparing the time series produced by the computer simulations, performed based on Ricker’s model parameters obtained from the census over two years in urban, farm and wild areas in Botucatu. The comparisons were made by using the concordance correlation coefficient (Lin 1989) to evaluate the reproducibility of the data (Table 3 A, B, C, D).

This coefficient evaluates the agreement between two readings by measuring the variation from the 45° line through the origin (the concordance line). Lin (1989) has shown that this method of assessing reproducibility is superior to comparison of coefficients, to the paired-*t* test, to regression, to the Pearson correlation, and to intraclass correlation (Zar 1996).

Results

The deterministic dynamics for both *L. eximia* and *C. albiceps* resulted in a monotonic stable equilibrium in response to their geometric growth rates, which are very similar and of a suitable magnitude to produce a monotonic stable equilibrium (Figs. 1A and B). The introduction of 1% migration between local populations provided the theoretical emergence of a population, from the farm area for *L. eximia* and from the wild area for *C. albiceps* (Figs. 2A and B). The simulations focused on stochastic carrying capacity resulted in global extinctions within a few generations for both *L. eximia* and *C. albiceps* (Figs. 3A and B). However, the simultaneous action of stochastic carrying capacity and migration increased significantly the persistence of the three populations in both species (Figs. 4A and 4B).

The stochasticity applied to the growth rate resulted in two persistent populations for both species. Nevertheless, the urban area populations of *C. albiceps* exhibited visibly higher-spectrum oscillations than those of *L. eximia* (Fig. 5A and B). The simultaneous stochasticity applied to growth rate and migration produced changes and a rescue of farm and wild populations in both *L. eximia* and *C. albiceps* (Figs. 5A and B, 6A and B). The simultaneous action of the stochasticity on carrying capacity and growth rate also resulted in global extinction of *C. albiceps* and *L. eximia* (Figs. 7A and B). However, the stochasticity applied to migration alone (Figs. 8A and B) maintained the three populations more stable and persistent compared to previous simulations.

Synchronous populations were frequently observed, mainly when the local migration was added to the simulations (Figs. 4A and B, 6A and B, 8A and B, 9A and B). Tables 3 A, B, C and D show the statistical analysis of spatial synchrony for *L. eximia* and *C. albiceps*. The concordance

correlation coefficients indicated important differences in the level of synchrony among the local populations of the two species. The synchrony analysis focused on the stochastic carrying capacity for the time series of *L. eximia* and *C. albiceps* showed no significant correlation between populations (Figs. 3A, B). The same result was obtained when the simultaneous stochastic carrying capacity and growth rate were analysed (Figs. 7A, B).

The synchrony analysis for migration alone indicated significant correlations for all populations of *C. albiceps* (Table 3A), and non-significant correlations for populations of *L. eximia*. The population connection that showed the highest correlation for both *L. eximia* and *C. albiceps* was the “farm x wild” (Tables 3B, C, D). Higher synchrony was found in the analysis focused on simultaneous stochastic carrying capacity and migration, than in that of stochastic growth rate and migration, for both species (Tables 3B, C, D). Most of the cases indicated that the simultaneous stochasticity for all parameters, i. e., for K , r and m , produces more synchrony than the stochastic carrying capacity and the stochastic growth rate alone, for both species (Tables 4 B, C, D).

Discussion

The deterministic analysis showed that both *L. eximia* and *C. albiceps* exhibit a stable equilibrium when analysed by the Ricker model. These results were certainly influenced by their growth rates estimated from the time series over 24 months. Generally, calliphorid species reach very high abundances in tropical areas (Souza and Linhares 1997), often with wide variability in population size (Serra et al. 2006). In this study, however, the low abundance of flies was probably the factor responsible for the magnitude of the growth rates.

The population dynamics of *L. eximia* was recently investigated by employing a density-dependent mathematical model developed by Prout & McChesney (1985), and the results suggest that this species exhibits a monotonic stable equilibrium, as seen in the current study (Silva et al. 2003). On the other hand, the population dynamics of *C. albiceps* was analysed by the same model and revealed a two-point limit cycle (Godoy et al. 2001). Nevertheless, the eigenvalue, the parameter that analyses the stability of the population equilibrium, obtained for *C. albiceps* was very close to 1

(Godoy et al. 2001), suggesting that the system may be susceptible to changes in stability in response to variations in parameter values. This includes possible changes from a limit cycle to a monotonic stable equilibrium, as observed in the current investigation (Godoy et al. 2001).

The frequency of *C. albiceps* and *L. eximia* differed in the three areas. *Chrysomya albiceps*, according to the deterministic simulations, tends to appear much more in urban areas and more rarely in farm areas; whereas *L. eximia*, in spite of being more frequent in urban areas, appears more occasionally in wild areas. Both species have been frequent in Brazil, in spite of exhibiting different abundances (Carvalho and Linhares 2001; Carvalho et al. 2004); and an occasional absence of them may be influencing their growth rate estimates, leading to the results found in the simulations. It is important to remember that the results obtained from simulations are merely projections generated from population numbers obtained from local time series. They should be viewed as theoretical possibilities, which may help us to understand how the population dynamics can play out under specific initial conditions, as for example, the numbers observed in the time series.

A rescue effect was observed after applying a very low migration rate ($m = 0.01$) for both *L. eximia* and *C. albiceps*. This effect is clearly seen in Figures 2A and B, which show the presence of farm and wild populations for *L. eximia* and *C. albiceps* respectively, not observed previously, in the simulations without migration. The rescue effect was recently observed in a similar study performed by Castanho et al. (2006), by applying a theoretical analysis in blowfly populations, using fuzzy subset approach. In some cases, the subpopulation of a species in a given environmental patch may fluctuate in size due to stochastic effects, especially when the population is small, leading it to local extinction (Akçakaya et al. 1999). However, a local extinction can be prevented by occasional immigrants arriving from neighboring populations (Gotelli, 1995).

Of all the parameters analysed in this study, K showed the highest negative impact in terms of population persistence in response to the stochasticity. This result was probably influenced by the K spectrum, leading the two species to global extinction within a few generations. Its negative effect can also be observed in the investigation, when the simultaneous stochastic action of r and K took place (see Figures 3AB and 7AB).

A clear effect of spatial synchrony on the populations of the two species was observed when the stochastic migration was added to the analyses, mainly observing the connection between populations of farm and wild areas. The possibility of synchrony between the populations of these areas is not surprising because they are very near to each other, facilitating the movements of the blowflies between patches. These results suggest that migration can exert a strong synchronous effect on the populations. Similar results were obtained by Serra et al. (2006), studying a two-patch metapopulation model applied to population growth of blowflies. However, in the study by Serra et al. (2006), the model employed incorporated parameters estimated in the laboratory.

The existence of synchrony is particularly significant to such systems, because it is directly related to the likelihood of global extinction (Heino et al. 1997). The more spatially synchronous a metapopulation is, the shorter is its expected persistence time. The reason for this is straightforward: if all local populations fluctuate in unison, then when one goes extinct, all others are likely to suffer the same fate; if spatial synchrony is low, some local populations are likely to be abundant and will serve to re-establish extinct populations (Heino et al. 1997).

Identification of the causes of synchrony is often difficult (Bascompte and Sole 1998). The synchronising effect of regional stochasticity has been observed in a variety of nonlinear population models as well (Haydon and Steen 1997; Kendall et al. 2000). In most systems, this effect, named Moran (Moran 1953), is thought to be the result of random but synchronous weather influences acting on spatially disjunct populations (Koenig 2002).

Demographic and environmental stochasticity can also strongly affect local population dynamics and the synchrony between the populations (Palmqvist and Lundberg 1998). Several studies using stochastic models have shown that the carrying capacity and environmental stochasticity play an essential role in population persistence (Gabriel and Bürger 1992). Theoretical studies have shown that population persistence in patchy environment results from an interaction between local density-dependence, dispersal and spatial heterogeneity (Chesson 1981). Negative density-dependence may cause populations to increase when individuals are rare, whereas positive density-dependence may cause populations to go extinct when individuals are rare (Amarasekare 1998). Stochasticity can also

reveal underlying deterministic patterns, and may show that subtle temporal patterns associated with deterministic chaos can indeed make themselves manifest (King et al. 2004).

Some theoretical studies have argued that asynchronous local populations have smaller risks of global extinction than do synchronous local populations, and hence are of particular concern in conservation (Hanski 1999). Specifically in the case of introduced and native blowflies, comparison of the results of this study with the abundance of blowflies recently censused in Brazil, suggests that if synchrony has taken place here it has had a weak influence on the persistence of flies. This conclusion is based on recent evaluations showing that the distributions of introduced and native blowflies in the last census, as well as their population sizes, have increased in the last 30 years (Carvalho et al. 2000). However, in the long term, synchrony can produce significant changes in the abundance of blowflies.

The results obtained by Serra et al. (2006) made evident the importance of spatial structure in a perspective of demographic stochasticity. They explored the stochastic population dynamics of *C. albiceps*, *C. megacephala*, *C. putoria*, *C. macellaria* and *L. eximia* by combining a density-dependent growth model with a two-patch metapopulation model. Surprisingly, *L. eximia* and *C. albiceps* were the species most susceptible to the risk of local extinction, and *C. macellaria*, *C. megacephala* and *C. putoria* exhibited the lowest risk of extinction (Serra et al. 2006).

In all simulations performed here, lower population sizes for *L. eximia* were found compared to *C. albiceps*. These results may suggest a better metapopulation performance for *C. albiceps*, an intraguild predator species (Faria et al. 1999; Rosa et al. 2006), which has appeared as the most frequent calliphorid species in several field studies (Carvalho et al. 2000; Carvalho & Linhares 2001; Carvalho et al. 2004). Nevertheless, *L. eximia* has exhibited qualities of a species able to resist a biological invasion by the three species of the genus *Chrysomya*, possibly by its habit to arrive in carcasses before the other species (Smith, 1986). This behaviour could be viewed as a temporal refuge that positively influences its performance. The analysis in the present study did not consider interspecific interactions. However, it gives us a useful insight into the theoretical probable causes of the possible specific dynamics behaviour in blowfly populations.

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Table 1 Abundance and distribution of calliphorid flies by species and area over two years

Month	<i>C. albiceps</i>			<i>L. eximia</i>		
	Urban	Farm	Wild	Urban	Farm	Wild
Mar	10	1	0	9	0	1
Apr	3	0	0	16	0	2
May	5	0	0	2	0	0
June	7	0	0	13	0	1
July	0	0	0	0	2	0
Aug	0	0	0	0	0	1
Sep	0	0	0	1	0	0
Oct	1	0	0	2	0	1
Nov	86	13	3	6	3	1
Dec	4	0	1	8	0	0
Jan	2	0	0	8	0	1
Feb	0	0	0	2	0	0
Mar	1	0	0	7	0	0
Apr	1	0	0	1	0	0
May	0	0	0	0	0	1
June	0	0	0	3	1	0
July	0	0	0	1	0	0
Aug	0	0	0	0	0	0
Sep	0	0	0	0	0	0
Oct	0	0	0	1	0	0
Nov	0	0	0	1	0	1
Dec	0	0	0	0	0	0
Jan	0	0	0	3	0	0
Feb	0	0	0	4	1	0
Σ	120	14	4	88	7	10

Table 2 Parameters used for simulations in the three-patch model

Urban area					
	r_{mean}	r_{max}	r_{min}	K_{max}	K_{min}
<i>L. eximia</i>	0.91	0.95	0.87	16	0
<i>C. albiceps</i>	0.74	0.95	0.53	86	0
Farm area					
	r_{mean}	r_{max}	r_{min}	K_{max}	K_{min}
<i>L. eximia</i>	1.26	1.52	1	3	0
<i>C. albiceps</i>	0.83	1	0.66	13	0
Wild area					
	r_{mean}	r_{max}	r_{min}	K_{max}	K_{min}
<i>L. eximia</i>	0.83	1	0.66	2	0
<i>C. albiceps</i>	1	1	1	3	0

r : geometric growth rate, K : carrying capacity, max: maximum, min: minimum

Table 3A Synchrony analysis in the time series of theoretical populations of *C. albiceps* under stochastic migration (m)

Statistics	Correlation between populations of <i>C. albiceps</i> under stochastic migration (m)		
	Urban x Farm	Urban x Wild	Farm x Wild
r_c	0.107	0.073	0.65
L1	0.390	0.322	0.741
L2	-0.175	-0.175	0.560
$r_{\text{(Pearson)}}$	0.640	0.608	0.811
L1	0.363	0.317	0.636
L2	0.813	0.795	0.906
F^*	4.561	4.112	9.597

- $F_{(0.05, 2) (28, 28)} = 2.13$, L_1 and L_2 : confidence intervals

Table 3B Synchrony analysis in the time series of theoretical populations of *L. eximia* and *C. albiceps*

Statistics	<i>L. eximia</i> (K and m)			<i>C. albiceps</i> (K and m)		
	Urban x Farm	Urban x Wild	Farm x Wild	Urban x Farm	Urban x Wild	Farm x Wild
r_c	0.473	0.509	0.939	0.61	0.502	0.894
L1	0.641	0.653	1.28	0.655	0.656	0.994
L2	0.304	0.364	0.599	0.565	0.348	0.794
$r_{\text{(Pearson)}}$	0.884	0.9	0.942	0.911	0.938	0.948
L1	0.768	0.798	0.88	0.82	0.873	0.892
L2	0.944	0.951	0.972	0.957	0.97	0.975
F^*	16.305	19.058	33.588	21.559	31.65	37.57

albiceps under simultaneous stochastic carrying capacity (K) and migration (m)

Table 3C Synchrony analysis in the time series of theoretical populations of *L. eximia* and *C. albiceps* under simultaneous stochastic growth rate (r) migration (m)

Statistics	<i>L. eximia</i> (r and m)			<i>C. albiceps</i> (r and m)		
	Urban x Farm	Urban x Wild	Farm x Wild	Urban x Farm	Urban x Wild	Farm x Wild
r_c	0.07	0.072	0.618	0.119	0.109	0.757
L1	0.327	0.309	0.845	0.401	0.39	1.02
L2	-0,186	-0,165	0.391	-0,162	-0,172	0.493
$r_{\text{(Pearson)}}$	0.648	0.561	0.627	0.632	0.639	0.781
L1	0.375	0.251	0.344	0.351	0.362	0.584
L2	0.818	0.767	0.805	0.808	0.813	0.89
F^*	4.691	3.561	4.365	4.428	4.555	8.133

Table 3D Synchrony analysis in the time series of theoretical populations of *L. eximia* and *C. albiceps* under simultaneous stochastic carrying capacity (K), growth rate (r) and migration (m)

Statistics	<i>L. eximia</i> (K , r and m)			<i>C. albiceps</i> (K , r and m)		
	Urban x Farm	Urban x Wild	Farm x Wild	Urban x Farm	Urban x Wild	Farm x Wild
r_c	0.665	0.661	0.922	0.701	0.666	0.912
L_1	0.778	0.758	1.263	0.83	0.737	1.227
L_2	0.551	0.565	0.582	0.573	0.594	0.597
$r_{\text{(Pearson)}}$	0.938	0.955	0.923	0.935	0.976	0.924
L_1	0.873	0.907	0.843	0.868	0.949	0.846
L_2	0.970	0.978	0.963	0.969	0.988	0.963
F^*	31.615	44.10	25.07	30.147	82.72	25.579

Figures

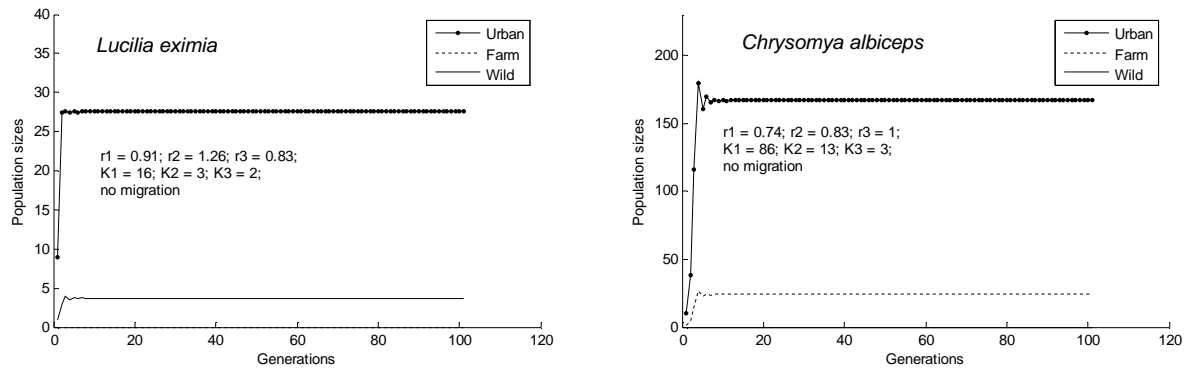


Fig. 1A-B Evolution of population sizes across generations obtained from simulations with a deterministic three-patch metapopulation model for non-migrant populations of *Lucilia eximia* (A) and *Chrysomya albiceps* (B).

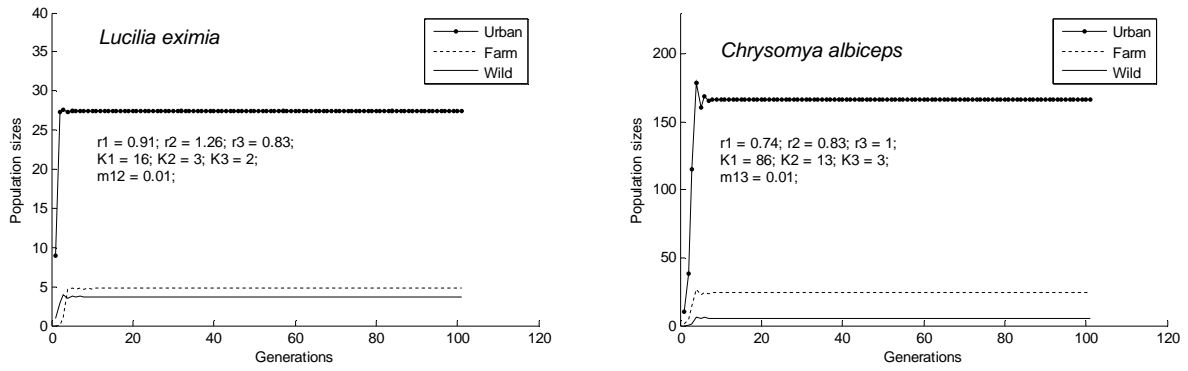


Fig 2A-B Evolution of population sizes across generations obtained from simulations with a deterministic three-patch metapopulation model for migrant populations of *Lucilia eximia* (A) and *Chrysomya albiceps* (B).

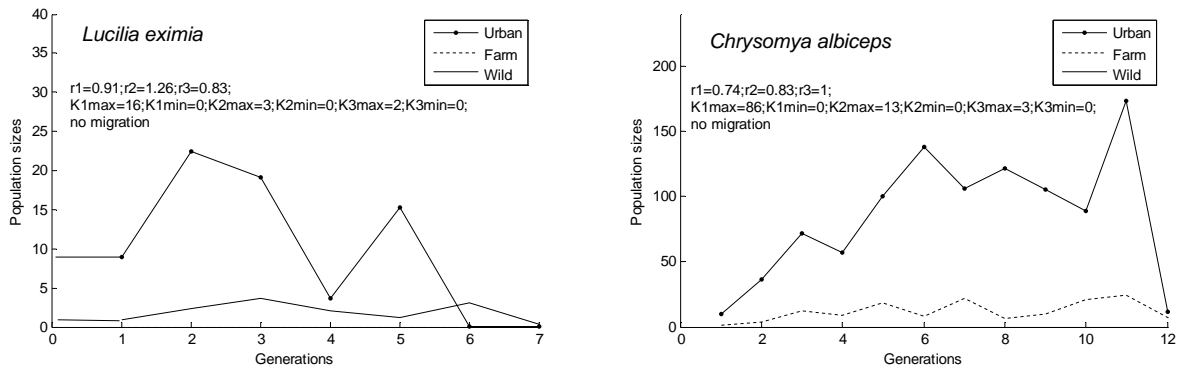


Fig 3A-B Metapopulation persistence obtained from simulations with stochastic carrying capacity for non-migrant populations of *Lucilia eximia* (A) and *Chrysomya albiceps* (B).

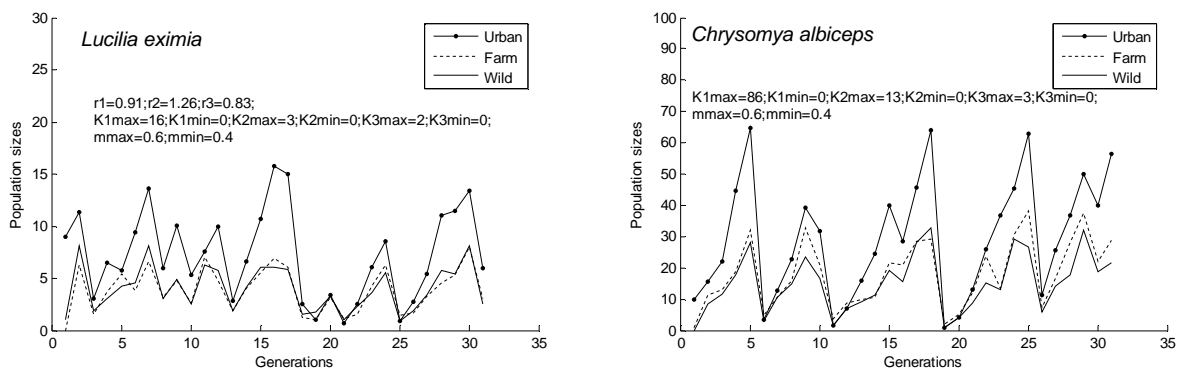


Fig 4A-B Metapopulation persistence obtained from simulations with simultaneous stochastic carrying capacity and migration for *Lucilia eximia* (A) and *Chrysomya albiceps* (B).

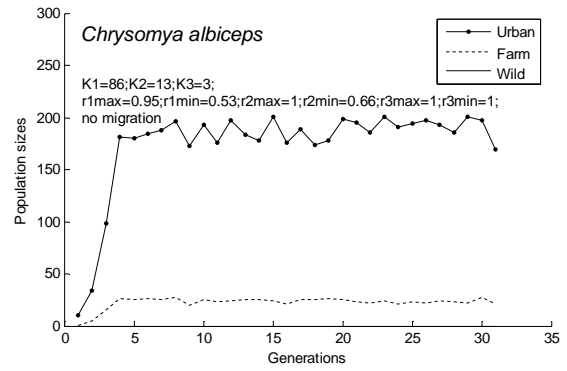
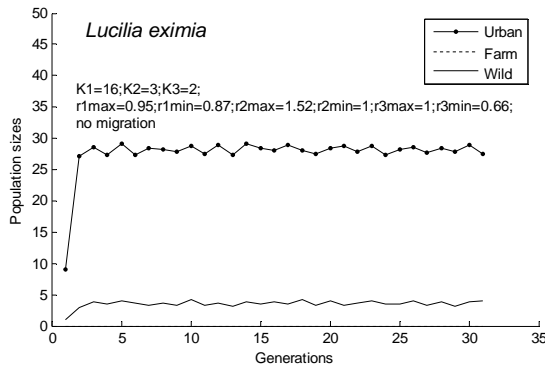


Fig 5A-B Metapopulation persistence obtained from simulations with stochastic geometrical

growth rate for non-migrant populations of *Lucilia eximia* (A) and *Chrysomya albiceps* (B)

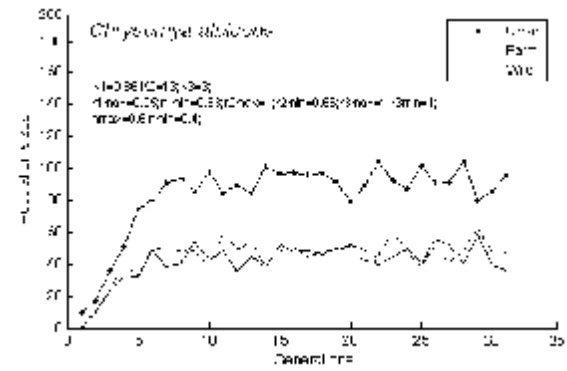
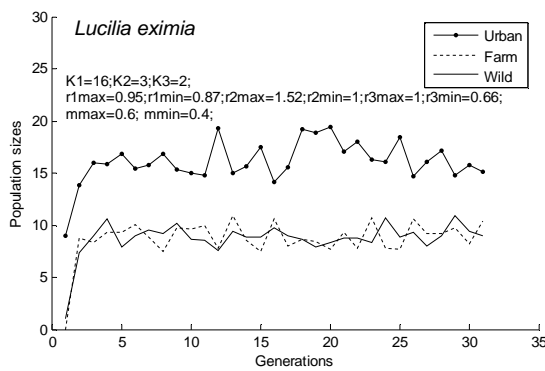


Fig 6A-B Metapopulation persistence obtained from simulations with simultaneous stochastic

geometrical growth rate and migration for *Lucilia eximia* (A) and *Chrysomya albiceps* (B)

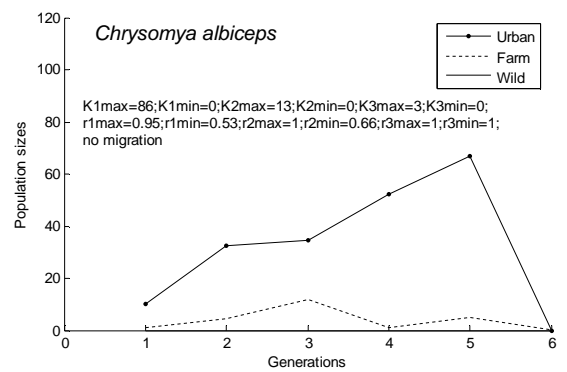
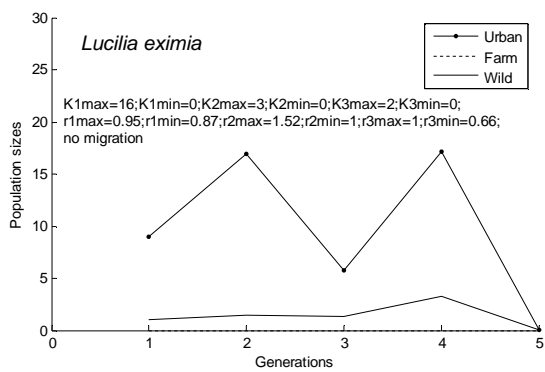


Fig 7A-B Metapopulation persistence obtained from simulations with simultaneous stochastic

carrying capacity and geometrical growth rate for non-migrant populations of *Lucilia eximia* (A) and *Chrysomya albiceps* (B)

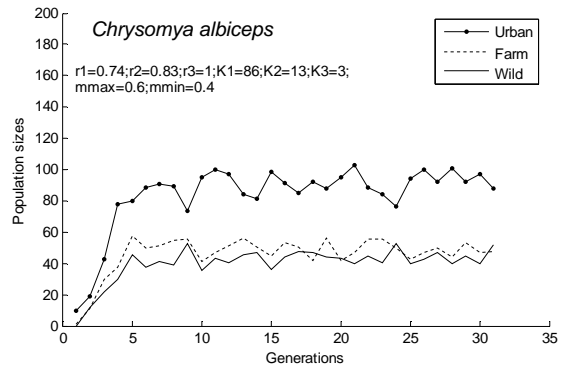
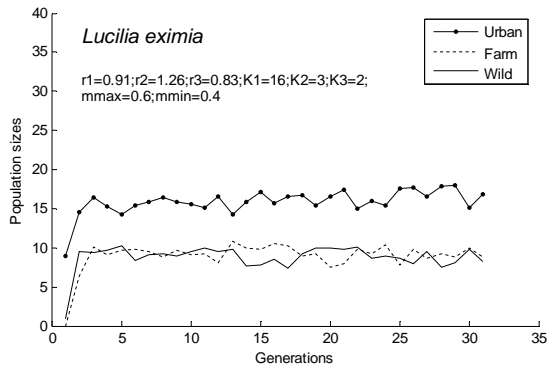


Fig 8A-B Metapopulation persistence obtained from simulations with stochastic migration for

Lucilia eximia (A) and *Chrysomya albiceps* (B).

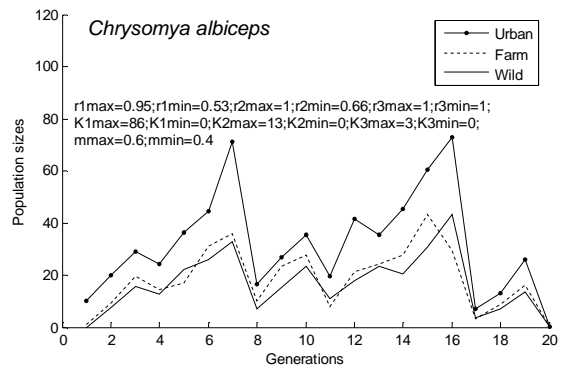
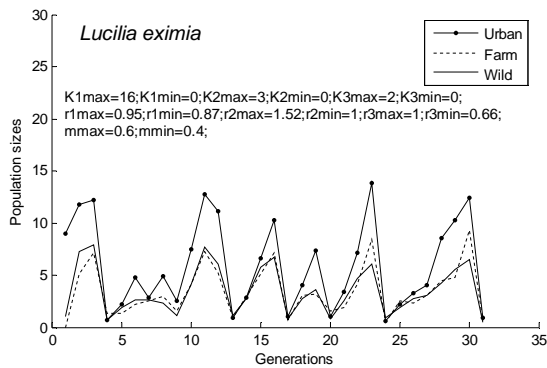


Fig 9A-B Metapopulation persistence obtained from simulations with simultaneous stochastic

carrying capacity, geometrical growth rate and migration for *Lucilia eximia* (A) and *Chrysomya albiceps* (B)

Molecular phylogeny in exotic and native blowflies of forensic importance in Brazil, based on mitochondrial DNA sequences

Abstract A molecular phylogeny analysis was performed on blowfly species of forensic importance. 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, ecological, and forensic contexts.

Keywords Molecular analyses • phylogeny • blowflies • mtDNA • COI

Introduction

Forensic entomology has been applied mainly to estimate the postmortem interval (PMI) based on the developmental rates and the successional ecology of specific insects that feed on carcasses [9]. Insects and other invertebrates feed on carrion in a successional manner, dependent on the state of decomposition. Blowflies are the first to colonise a body [3]. Generally the time since the corpse was exposed to the insects is estimated by using eggs, third instar larvae and adults, because it is easier to identify the species and hence the respective duration of the life cycle [3, 38]. The recognition of the species involved, the pattern and time of arrival at the scene of the adults, and subsequently the eggs and larvae, together with knowledge of their development rates can give an indication of the time of death [2].

In forensic entomology, information is essential not only on the developmental stages of the insects found on a body, but also on their identity [1]. For some groups of insects, differentiation at the larval stages using morphological criteria is still not possible. Time-consuming rearing of the larvae to adults for identification may delay a criminal investigation, or cause significant problems when rearing fails. Under these circumstances, species identification based on genetic examination is an option. Flies are the most important insects in forensic entomology, and therefore genetic research has focused on Diptera [21, 45, 47, 48]. Modern DNA techniques are contributing to the rapid and authoritative identification of necrophagous insects. Phylogenetic analysis using the reference data here presented can determine the species of a specimen collected from a human corpse anywhere in Brazil. This approach appears to be reliable for identifying

highly degraded tissue, as well as specimens collected from separated or nearby geographic locations.

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* [6, 18, 19]. 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 [45]. 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 [46]. 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 [46], on the other hand genetic variation in populations of *L. cuprina* and *L. sericata* is also possible [40].

Wells and Sperling (46) 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 by using mitochondrial DNA

as the basis for sequencing [21, 27, 45]. 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 [24].

Most literature in the field of forensic entomology has addressed the necrophagous fauna of Australia, Europe, and the United States. In South America, particularly in Brazil, forensic entomology has gradually received more attention [8, 39]. Until now, Brazilian researchers have focused on the succession of insects on animal carcasses [7, 8]. 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 forensically significant species. However, its usefulness has become evident, as several African insect species have been observed in South America [6, 18, 19]. Then, the objective of this study was to perform a molecular phylogeny analysis on blowfly species of forensic importance in localities separated by short distances.

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 that had been identified 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 [10, 33], in part because mtDNA rarely recombines and has a relatively rapid rate of nucleotide substitution [4].

2.1. Flies and materials

The specimens of *Chrysomya albiceps*, *C. megacephala*, *C. putoria*, *Lucilia eximia*, *Cochliomya macellaria*, and *Hemilucilia segmentaria* used in this study were 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), Gramado in the state of Rio Grande do Sul (29°24'17'' South, 50°52'35'' West), Presidente Prudente in the state of São Paulo (22°07'32'' South, 51°23'23'' West) and 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 X 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 [20]. A Zeiss Stemi 2000 (W_Pl 10x/23) was used to observe the characters. A data matrix was prepared for each specimen.

2.2 Genome DNA extraction

Total individual DNA was extracted from individual female flies, and amplified by PCR based on the universal primers reported by Wells & Sperling [46]. Each specimen was preserved in 70% ethanol.

2.3 PCR

The amplification reaction was carried out in a total volume of 25 μ l, with a final concentration of 10 x PCR buffer (Tris-HCl 200_mM pH 8.4; KCl 500_mM), 1.5 mM MgCl₂, 0.2_mM DNTPs (Invitrogen), 1U of Taq DNA polymerase (Pharmacia) and 1 μ M of each of the primers COI F and COI R. 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 94°C, 2 min at 37°C, and 1 min at 72°C followed by 35 repetitive cycles of 30_s at 94°C, 30_s at 50°C, and 1 min at 72°C, followed by an additional extension cycle at 72°C for 5 min. All amplifications were performed on a Whatman Biometra[®] (T gradient) thermocycler.

Aliquots of amplified products (8 μ l) 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”.

2.4 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, Foster City, California, U.S.A). Approximately 10 ng of purified DNA, for each sequencing reaction, was combined with 3.2 pmol 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.

2.5 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 [43] set to default parameters, with manual adjustments where necessary. Aligned sequences were analysed using the MEGA software package [23]. Methods of Distance (Neighbour-Joining – NJ) and Parsimony were used to construct the phylogenetic tree [36]. A phylogenetic tree was visualised using the TREEVIEW 1.4 program [30]. The bootstrap test was applied to estimate the confidence of branching patterns of the neighbour-joining tree [11].

2.6 Statistical analysis of mitochondrial haplotype frequencies

For each collection, the nucleotide sequence and frequency of each haplotype were entered into DnaSP v 3.5 [35]. 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).

2.7 GenBank accession numbers

The nucleotide sequences reported in this paper have the following GenBank accession numbers: CALB1 EF136633, CALB2 EF136634, LEXI3 EF136635, LEXI1136636, LEXI4 EF136637, LEXI2 136638, LEXI5 EF136639, LCUP EF136640, CMEG EF136641, CPUT EF136642 CMAC1 EF136643, CMAC3 EF136644, CMAC4 EF136645, CMAC2 EF136646 and HLUC EF136647.

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 ($\kappa = 22.895$) 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 of the percentage support for a grouping by randomly resampling the data.

The three species of the genus *Chrysomya* were grouped with high bootstrap support. At species level, specimens of *C. macellaria* and *L. eximia* formed single clusters with 100% support (Figure 1). Within the *L. eximia* clade considerable variation was evident, showing two other clusters with high support (Figure 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 colonisation of the Americas by *Chrysomya* species has reportedly led to reduction in the native fly fauna [6, 31]. The decrease in the genetic variability of *C. macellaria* populations

has been associated with the presence of *Chrysomya* [44]. However, the exact source of New World *Chrysomya* remains to be defined. Morphologically, calliphorids are generally easy to identify to subfamily level, and the molecular data presented here support the separation of the Chrysomyinae and Luciliinae.

Chrysomya albiceps and *C. rufifacies*, and *L. cuprina* and *L. sericata*, are recognised as being difficult to distinguish morphologically [40, 42, 46]. 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 [46]. In Latin America, where the distributions of these species overlap [42], 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 [5, 28] suggests that it would be important to investigate the evolutionary processes related to these facultative species. Stevens et al. [41] demonstrated divergent nuclear and mitochondrial phylogenies in hybrid *Lucilia* spp. Given the apparently great age (see below) of these subfamilies (Chrysomyinae 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 [12].

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 [26, 31] and has been reared from a wide variety of corpses, including pigs [39].

Introduced and native blowfly species have shown interesting differences in terms of dynamic behaviour in Brazilian populations [16]. A research programme was initiated 12 years ago in order to understand the process of invasion by blowflies in Brazil [14, 15, 16, 34, 37]. 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 [32], 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 [14, 15, 16, 37].

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 [14, 15, 16, 37]. 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* [13].

Lucilia eximia can apparently maintain a more stable population size than other calliphorid species, when facing environmental disturbances [37]. This conjecture can be explained by the lack of seasonal variations or particular habitat preferences in this species [29]. Linhares [25] 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 [22]. 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 [17]. 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.

Acknowledgements

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Table 1. Geographic origins of the specimens collected in the four areas.

Region	Specimens
Botucatu, SP	Lexi: 10; Lcup: 7; Cmeg: 15; Calb: 14; Hluc: 9; Cmac: 5; Calb: 6.
Presidente Prudente	Cput: 3; Cmeg:6.
Nova Andradina	Cmeg: 2; Calb: 17.
Gramado, RS	C meg: 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 position 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	115	116	118	121	124	130	133	136	148	151	154	157	160
<i>H. segmentaria</i>	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	A	C	A	T	A	A	A	T	T	A	C	T	C
<i>C. macellaria 1</i>	.	C	.	.	A	.	.	.	T	.	T	C	A	.	.	.	C	T	A	C	.	.	T	A	.	T	A	C	
<i>C. macellaria 2</i>	.	C	.	.	A	.	.	.	T	.	.	C	A	.	.	.	C	T	A	C	.	.	T	A	.	T	A	C	
<i>C. macellaria 3</i>	G	C	.	.	A	.	.	.	T	.	.	C	A	.	.	.	C	T	A	C	.	.	T	A	.	T	A	C	
<i>C. macellaria 4</i>	G	C	.	.	A	.	.	.	T	.	.	C	A	.	.	.	C	T	A	C	.	.	T	A	.	T	A	C	
<i>C. putoria</i>	.	.	T	T	G	.	.	T	C	A	.	A	.	.	.	T	.	A	.	T	.	C	A	G	T	.	T	
<i>C. megacephala</i>	.	.	T	T	A	.	.	C	.	T	.	.	A	.	A	C	T	T	.	.	A	G	T	.	.	A	.	T	.	T
<i>L. cuprina</i>	.	.	T	.	.	.	T	C	.	.	G	.	.	T	.	.	A	C	A	C	T	T	T	.	A	.	T	.	.	A	.	T	.	.
<i>C. albiceps 1</i>	.	.	T	T	A	T	.	.	.	T	.	.	A	.	A	C	T	T	.	T	A	.	T	.	C	A	.	.	.	T
<i>C. albiceps 12</i>	.	.	T	T	A	T	.	.	.	T	.	.	A	.	A	C	T	T	.	T	A	.	T	.	C	A	.	.	.	T
<i>C. albiceps 13</i>	.	.	T	T	A	T	.	.	.	T	.	.	A	.	A	C	T	T	.	T	A	.	T	.	C	A	.	.	.	T
<i>C. albiceps 14</i>	.	.	T	T	A	T	.	.	.	T	.	.	A	.	A	C	T	T	.	T	A	.	T	.	C	A	.	.	.	T
<i>L. eximia 1</i>	.	.	T	T	.	.	A	.	A	.	C	.	T	G	.	.	T	.	.	A	C	A	.	.	.	T	.	A	.	.	T	C	A	.	T	C	T	
<i>L. eximia 2</i>	.	.	T	T	.	.	A	.	A	.	C	.	T	G	.	.	T	.	.	A	C	A	.	.	.	T	.	A	.	.	T	C	A	.	T	C	T	
<i>L. eximia 3</i>	.	.	T	T	.	.	A	.	A	.	C	.	T	G	.	.	T	.	C	A	C	A	.	.	.	T	.	A	.	.	T	C	A	.	T	C	T	
<i>L. eximia 6</i>	.	.	T	T	.	G	.	A	.	A	.	C	.	T	G	.	.	T	.	C	A	.	A	.	.	.	T	.	A	.	.	T	C	A	.	T	C	T
<i>L. eximia 7</i>	.	.	T	T	.	G	.	A	.	A	.	C	.	T	G	.	.	T	.	C	A	.	A	.	.	.	T	.	A	.	.	T	C	A	.	T	C	T
	165	166	169	170	175	178	182	187	188	193	194	195	196	199	200	203	208	217	218	232	235	242	247	250	253	262	265	274	275	277	280	288	290	291	295	301	304	
<i>H. segmentaria</i>	A	C	T	C	T	A	C	C	T	T	A	G	A	G	A	C	A	C	G	T	A	T	T	C	C	T	C	A	G	G	A	G	C	G	C	C	T	
<i>C. macellaria 1</i>	.	T	.	.	.	T	T	T	T	G	T	.	.	G	C	.	.	.	A	T	T	.	
<i>C. macellaria 2</i>	.	T	.	.	.	T	T	T	T	G	T	.	.	G	C	.	.	.	A	T	T	C	
<i>C. macellaria 3</i>	.	T	.	.	.	T	T	T	T	G	T	.	.	G	C	.	.	.	A	T	T	C	
<i>C. macellaria 4</i>	T	T	T	T	G	T	.	.	G	C	.	.	.	A	T	T	C	
<i>C. putoria</i>	T	.	.	.	T	T	T	C	.	G	.	T	.	.	T	G	T	A	A	T	.	.		
<i>C. megacephala</i>	T	.	.	.	T	T	T	C	.	T	T	G	T	.	C	A	T		
<i>L. cuprina</i>	.	.	T	C	.	T	T	.	.	.	C	T	.	.	T	.	T	G	.	A	A		
<i>C. albiceps 1</i>	T	.	A	T	.	T	T	T	C	.	.	A	T	A	.	T	.	T	A	.	T	.	T	.	T	.	.	T	.	C		
<i>C. albiceps 12</i>	T	.	A	T	.	T	T	T	C	.	.	A	T	A	.	.	.	T	A	.	T	.	T	.	T	.	A	T	T	.	C	
<i>C. albiceps 13</i>	T	.	A	T	.	T	T	T	C	.	.	A	T	A	.	.	.	T	A	.	T	.	T	.	A	T	T	.	.	T	.	C
<i>C. albiceps 14</i>	T	.	A	T	.	T	T	T	C	.	.	A	T	A	.	.	.	T	A	.	T	.	T	.	T	T	.	.	T	.	C	
<i>L. eximia 1</i>	.	.	A	T	.	T	.	.	.	C	.	C	.	A	.	T	.	.	A	.	G	C	A	T	.	C	.	G	.	C	T	.	
<i>L. eximia 2</i>	.	.	A	T	.	T	.	.	.	C	.	C	.	A	.	T	.	.	A	.	.	C	A	T	.	C	.	G	.	C	T	.	
<i>L. eximia 3</i>	.	.	A	T	.	T	.	.	.	C	.	C	.	A	.	T	.	.	A	.	.	C	A	T	.	C	.	G	.	C	T	.	
<i>L. eximia 6</i>	.	T	A	T	.	T	.	.	.	C	.	C	.	A	.	T	.	.	A	.	.	C	A	T	.	C	.	G	.	C	.	T	
<i>L. eximia 7</i>	.	T	A	T	.	T	.	.	.	C	.	C	.	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
<i>C. albiceps</i>	31	4	2	0,488	0,488	0,00157
<i>L. eximia</i>	10	7	5	0,8000	2,97778	0,00961
<i>L. cuprina</i>	7	0	1	0	0	0
<i>C. megacephala</i>	25	0	1	0	0	0
<i>C. putoria</i>	3	0	1	0	0	0
<i>C. macellaria</i>	5	3	4	0,9000	1,6000	0,00516
<i>H. lucilia</i>	9	0	1	0	0	0

Figure legend

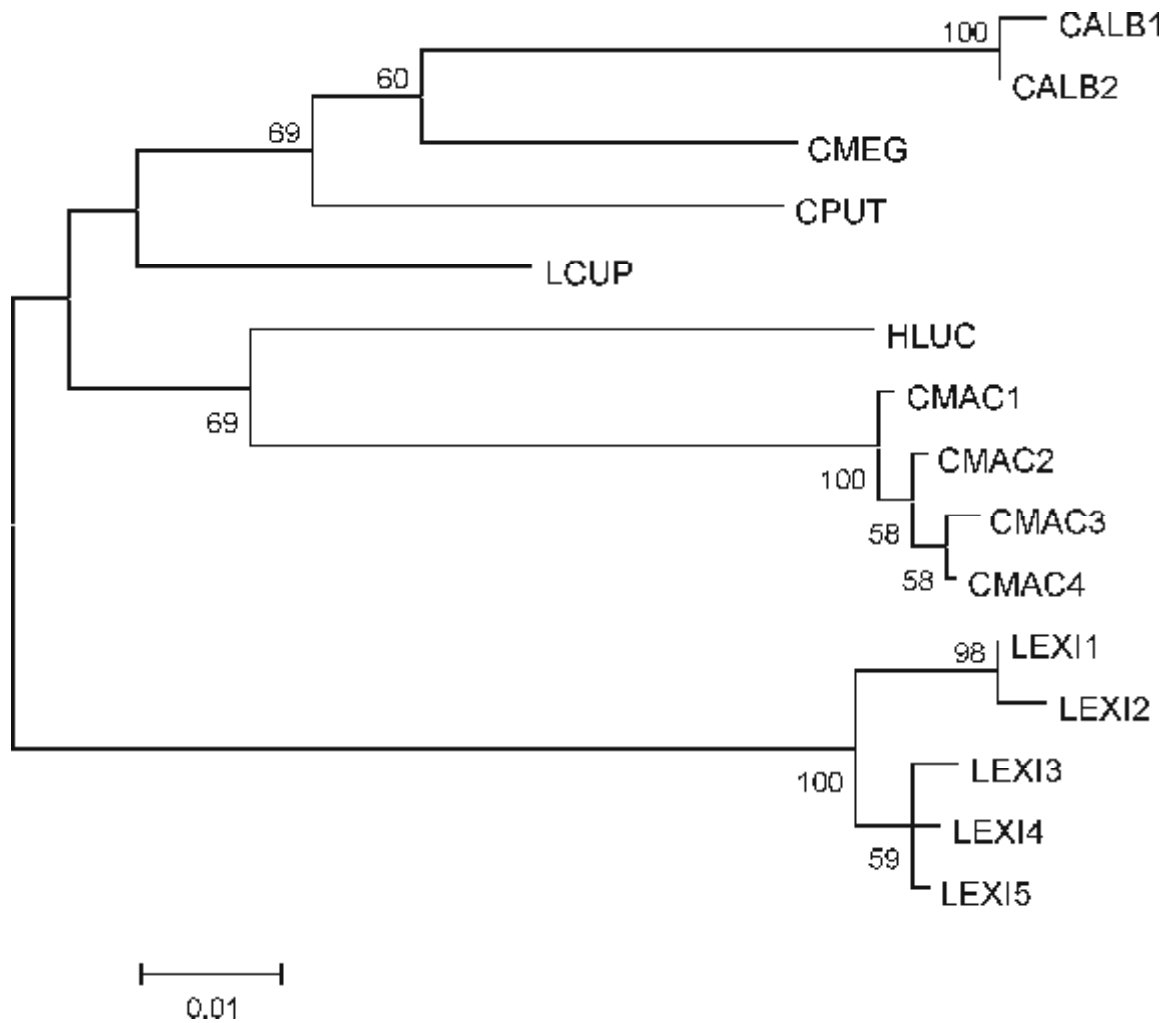


Figure 1: Blow fly phylogeny based on Neighbour-Joining genetic distances among populations in Brazil. Bootstrap values over 50%, based on 1,000 permutations, are indicated on the nodes.

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