Assessing genetic and morphological variation in populations of Eastern European *Lucilia sericata* (Diptera: Calliphoridae)

**Anna V. DiaKova**1,*, **Dmitry M. Schepetov**2, **Nadezhda Y. Oyun**1,3,4, **Anatole I. Shatalkin**5 and **Tatiana V. Galinskaya**1,6,*

1 Entomology Department, Biological Faculty, Lomonosov Moscow State University, Leninskie gory 1–12, Moscow 119234, Russia; e-mails: annidakova@yandex.ru, nad_oyun@mail.ru, nuha_1313@list.ru
2 National Research University Higher School of Economics, Moscow, Russia; e-mail: schep@mail.ru
3 Vavilov Institute of General Genetics, Russian Academy of Sciences, Gubkina St. 3, Moscow 119991, Russia
4 Martinovsky Institute of Medical Parasitology, Tropical and Vector-Borne Diseases, Sechenov University, Malaya Pirogovskaya St. 20-1, Moscow 119435, Russia
5 Zoological Museum, Lomonosov Moscow State University, Bol’shaya Nikitskaya St. 6, Moscow 125009, Russia; e-mail: shatalkin@mail.ru
6 Scientific-Methodological Department of Entomology, All-Russian Plant Quarantine Center, Pogranichnaya 32, Bykovo 140150, Russia

**Key words.** Diptera, Calliphoridae, *Lucilia sericata*, forensic entomology, microsatellite, population genetic structure, blowfly

**Abstract.** The population structures of different species of Calliphoridae flies are highly diverse at different locations. We investigated populations of the Eastern European *L. sericata* using chaetotaxy and eight microsatellite loci. Our results strongly indicate that a panmictic population of *L. sericata* exists in the area studied, possibly with a high rate of intra-population gene flow. Analysis of chaetotaxy also supports the panmictic population hypothesis.

**INTRODUCTION**

*Lucilia sericata* Meigen, 1826 (Diptera: Calliphoridae), or the common green bottle fly, is widespread and usually abundant. This species is forensically important, and often used for estimating time since death (Postmortem Interval) (Karabey & Sert, 2014). Moreover, their larvae are facultative parasites, capable of developing in body tissues of mammals, which causes myiasis (Nelson & Rice, 1956; Hall & Wall, 1995). This disease often affects sheep, causing significant economic loss and making *L. sericata* an important veterinary pest (French et al., 1994).

The population structures of different species of flies belonging to the family Calliphoridae are very diverse. Some species are panmictic over a wide geographic range, as is *Phormia regina* Meigen, 1826 (Diptera: Calliphoridae) in USA (Picard & Wells, 2009; Jordaens et al., 2013). Some appear to have distinct well separated populations, for example *Lucilia cuprina* Wiedemann, 1830 (Diptera: Calliphoridae) in Australia (Clarke & McKenzie, 1987). Different types of population structure may exist even in one species of Calliphoridae: e.g., *Cochliomyia hominivora* Coquerel, 1858 (Diptera: Calliphoridae) has a distinct population structures in the Caribbean, even within islands (Torres et al., 2004; Torres & Azeredo-Espin, 2005, 2009). However, clear evidence exist that the same species is panmictic in Uruguay (Lyra et al., 2005; Torres et al., 2007).

Different types of population structure may exist even in one species of Calliphoridae: e.g., *Cochliomyia hominivora* Coquerel, 1858 (Diptera: Calliphoridae) has a distinct population structures in the Caribbean, even within islands (Torres et al., 2004; Torres & Azeredo-Espin, 2005, 2009). However, clear evidence exist that the same species is panmictic in Uruguay (Lyra et al., 2005; Torres et al., 2007). As the population structure of Calliphoridae is often complex (Lyra et al., 2009; Rodrigues et al., 2009) and calliphorid species are important for forensic and veterinary science, studying them is both fundamentally important (Florin, 2001) and practical (Karabey & Sert, 2014). In this article, we present the results of the first calliphorid population study performed in Eastern Europe. In our research, we combine population genetics and a classical morphological approach. We selected, characterized and analyzed eight microsatellite loci. Microsatellites are a common tool used in DNA based population studies, because microsatellite loci are usually highly polymorphic, providing a wide range of alleles with a high level of heterozygosity. This makes them the method of choice for genetic studies of population structure and estimating gene flow between populations (Jarne & Lagoda, 1996; Li et al., 2004).

* Equal contributors.
Microsatellite analysis

Eight pairs of primers were designed by us using Websat software (Martins et al., 2009) and the _L. cuprina_ genome sequence (Anstead et al., 2015). One pair of primers was adapted from Florin & Gyllenstrand (2002) (Table 2).

PCR reactions were performed using the following protocol:

1. Four cycles: 40 s at 95°C, 90 s at 52°C (increasing 0.5°C a step up to 54°C), 60 s at 70°C.
2. 35 cycles: 40 s at 95°C, 60 s at 54°C (decreasing 1 s per step), 60 s at 70°C.

Amplifications for this study were done using a HS-Taq Kit (Evrogen, Moscow, Russia).

DNA was extracted from leg muscle following the protocol described by Galinskaya et al. (2016).

Population genetic analysis

We determined whether putative genetic segregation exists in the samples studied using STRUCTURE 2.0 software and by implementing the Bayesian algorithm for detecting population structure (Pritchard et al., 2000). Further we used an improved method described by Evanno et al. (2005) to detect the proper number of clusters (K). Allele frequencies were calculated using...
SPAGeDi software (Hardy et al., 2003). Effective population size was estimated using NeEstimator software (Do et al., 2014).

Morphological survey

The characters analyzed included: number of hairs on the posterior slope of the humeral callosity behind the basal setae (see 1 in Fig. 2A, B), number of hairs on the edge of the notopleuron behind the posterior notopleural seta (see 2 in Fig. 2A, B), number of setulae on the “quadrat” between the discal setae and anterior margin of scutellum (Fig. 2C). These characters were adapted from a study on *L. sericata*, *L. cuprina* and their hybrids (Williams & Villet, 2014). Statistical analysis was performed using STATISTICA 8.0 software (TIBCO Software Inc, Palo Alto, CA, USA).

Table 2. Microsatellite primers used in the genetic analysis of *Lucilia sericata*.

<table>
<thead>
<tr>
<th>Locus</th>
<th>5′-3′ sequence, Forward</th>
<th>5′-3′ sequence, Reverse</th>
<th>Repeat motif</th>
</tr>
</thead>
<tbody>
<tr>
<td>loc7</td>
<td>GGAAAAGGGGATGAAAGAGAGT</td>
<td>GTGAATCGTGTGGAAGGTTTT</td>
<td>(CA)$_5$</td>
</tr>
<tr>
<td>loc17</td>
<td>TGCTCAGGATAAACCCTACA</td>
<td>GAATACAAACCCTAACCCTCG</td>
<td>(GT)$_5$</td>
</tr>
<tr>
<td>loc19</td>
<td>TAGGAGCAAAGTTTGGACGA</td>
<td>TGACGTTAATATCGTCAGAC</td>
<td>(GT)$_2$</td>
</tr>
<tr>
<td>L2</td>
<td>TGTCACCDCCAGCTTGTAGCC</td>
<td>ACGTTAGGGAATATCCATGAG</td>
<td>(CAA)$_2$, CCA $(CAA)_2$, CCA $(CAA)_2$</td>
</tr>
<tr>
<td>AD1</td>
<td>GTGCTTAACCGTGTAGCTCAG</td>
<td>TAGAGAAGTTTGGTCAAGG</td>
<td>(TC)$_5$</td>
</tr>
<tr>
<td>AD6</td>
<td>CGAGTTTATGCTTCAGGC</td>
<td>CAGAGAAATTAGTCAGGAAAAC</td>
<td>(CA)$_5$</td>
</tr>
<tr>
<td>AD8</td>
<td>TGATCGTGCGGCTTGTAGCT</td>
<td>CTCAGTCCTACCAACTAAAATG</td>
<td>(TG)$_5$</td>
</tr>
<tr>
<td>AD9</td>
<td>CCATTTTATAGCGGTTTCCAG</td>
<td>CAACAAGAGTATCTGACAACCA</td>
<td>(AC)$_5$</td>
</tr>
<tr>
<td>AD10</td>
<td>GAATCTCAAACGCTCCATAG</td>
<td>AAGTATTTCAAGCACAATCC</td>
<td>(AC)$_5$</td>
</tr>
</tbody>
</table>
RESULTS

All the loci used in the present study were highly polymorphic (Table 3). The STRUCTURE simulation indicated no significant correlation between locations and potential clusters (Fig. 3). The most likely number of clusters was one, which clearly indicates the absence of any population structure.

The effective population size was estimated as “infinite”. The authors of the original software suggest that this result indicates an absence of evidence of genetic drift due to a finite number of parents in the samples.

Significant variability was recorded in the chaetotaxy characters. Using two-way ANOVA, we detected sexual dimorphism based on a difference in the number of setulae on the scutellum in males and females (Table 4). No groups with distinct differentiation were found for any of the three characters analyzed. Furthermore, we detected no correlation between sampling location and the chaetotaxy characters studied.

DISCUSSION

Results of our genetic analysis indicate that there is a panmictic population of L. sericata in the area studied, with putatively a high rate of gene flow within this population. We assume that the ability of L. sericata to migrate long distances and their high fertility outweighs existing genetic drift and effects of geographical distances. The assumption that genetic drift is relatively negligible is also supported by the results of the effective population size estimations, i.e. the “infinite” number of breeders. However, it is also possible that overlapping generations or a sampling error could have led to similar results (Waples et al., 2014). The chaetotaxy analysis also strongly supports our panmictic population hypothesis.

In previous studies, evidence of L. sericata panmixy in part of the range of this species in the United States is reported by Picard & Wells (2010). No correlation between location and population structure was established. However, flies coming to bait over a short period of time were closely related. In earlier work, Stevens and Wall also failed to demonstrate significant genetic differences between geographically separated worldwide populations of L. sericata (Stevens & Wall, 1997).

A detailed study of populations of Fletcherimyia fletcheri Aldrich, 1916 (Diptera: Sarcophagidae) revealed significantly different results, yet F. fletcheri belongs to the same superfamily, Oestridae (Rasic & Keyghobadi, 2012). This species oviposits less than 10 eggs and the larvae develop inside the leaves of an insectivorous plant, Sarracenia purpurea. However, it is not surprising that with such a strong connection with their habitat and such a low fertility populations of F. fletcheri have a distinct structure even over small ranges, as isolation by distance is reported over...
distances of 10–26 km. In comparison, the distance sufficient for isolation for populations of *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae) is estimated as more than 100 km (Chakrabarti et al., 2010). It should be noted that *M. domestica* is polyphagous and oviposits about as many eggs as *L. sericata* (100–150 in *M. domestica* compared to 150–200 in *L. sericata*).

Considering the examples mentioned above, we conclude that the population structure of flies is highly influenced by aspects of their biology, especially fertility, migration capacity, dependence on food sources and abundance of food. In the case of *L. sericata* our findings, as well as earlier studies, indicate a tendency towards panmixy in different parts of the world. This should be taken into account in future research on this species, as well as in forensic and veterinary studies.

**ACKNOWLEDGEMENTS.** We thank D.M. Astakhov (Department of Biology, Institute of Natural Sciences, Volgograd State University), S.M. Tsurikov (Severtsov Institute of Ecology and Evolution) and S.N. Lysenkov (Faculty of Biology, Department of Evolutionary Biology, Lomonosov Moscow State University) for providing specimens of flies, and V.G. Tambovtseva (Koltsov Institute of Developmental Biology, Russian Academy of Sciences) for her help with molecular analyses. Examination and photographing of external morphological elements was performed using equipment obtained with the support of the Russian Science Foundation (project number 14-50-00029, “Scientific bases of the national biobank, the depository of living systems”). The molecular part of the work (plastic, reagents, sequencing and data analysis) was funded by the Russian Foundation for Basic Research (project number 16-04-01358-a). The molecular part of work was performed using equipment of the Core Centrum of the Koltsov Institute of Developmental Biology, Russian Academy of Sciences. Bioinformatic analysis and computer modeling in this research was conducted within the framework of the Basic Research Program at the National Research University Higher School of Economics (HSE) and supported within the framework of a subsidy granted to the HSE by the Government of the Russian Federation for the implementation of the Global Competitiveness Program.

**REFERENCES**


