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ORIGINAL ARTICLE

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

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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 hominivorax* 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).

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Fig. 1. Map showing the sites where *Lucilia sericata* were collected, indicated by black dots. Grey dots indicate some of the larger cities.

Chaetotaxy, on the other hand, is a method based on set of characters widely used in the morphological approach to systematics, evolution and population studies. We compared the results of the chaetotaxy study with those of the genetic analysis.

MATERIALS AND METHODS

Specimen collection

Adult flies of *L. sericata* were collected on decaying organic matter at seven locations (Table 1, Fig. 1) and stored in 95% ethanol, in a freezer at −20°C.

DNA extractions

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

Table 1. Geographic locations, dates of collection and numbers of *Lucilia sericata* collected.

Location	Coordinates	Date of collection	No. of spec.
Yevpatoria	45°19′N, 33°36′E	28.vi.16, 30.vi.16	17
Koktebel	44°96′N, 35°24′E	3.–8.vii.16	23
Zuya	45°05′N, 34°31′E	2.vii.16	11
Sevastopol	44°61′N, 33°52′E	10.–14.vii.16	13
Aleksin	54°50′N, 37°E	4.ix.16	15
Volgograd	48°25′N, 43°2′E	21.viii.16	9
Lipetsk	52°61′N, 39°59′E	21.vii.16	14

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).

Forward primers were labelled on 5' end with TAMRA, FAM or R6G fluorescent marker. Product length was measured by capillary gel-electrophoresis on an Applied Biosystems Genetic Analyzer 3500 (Thermo Fisher Scientific Inc, Waltham, MA, USA). The results were then processed using GeneMarker software (Hulce et al., 2011).

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

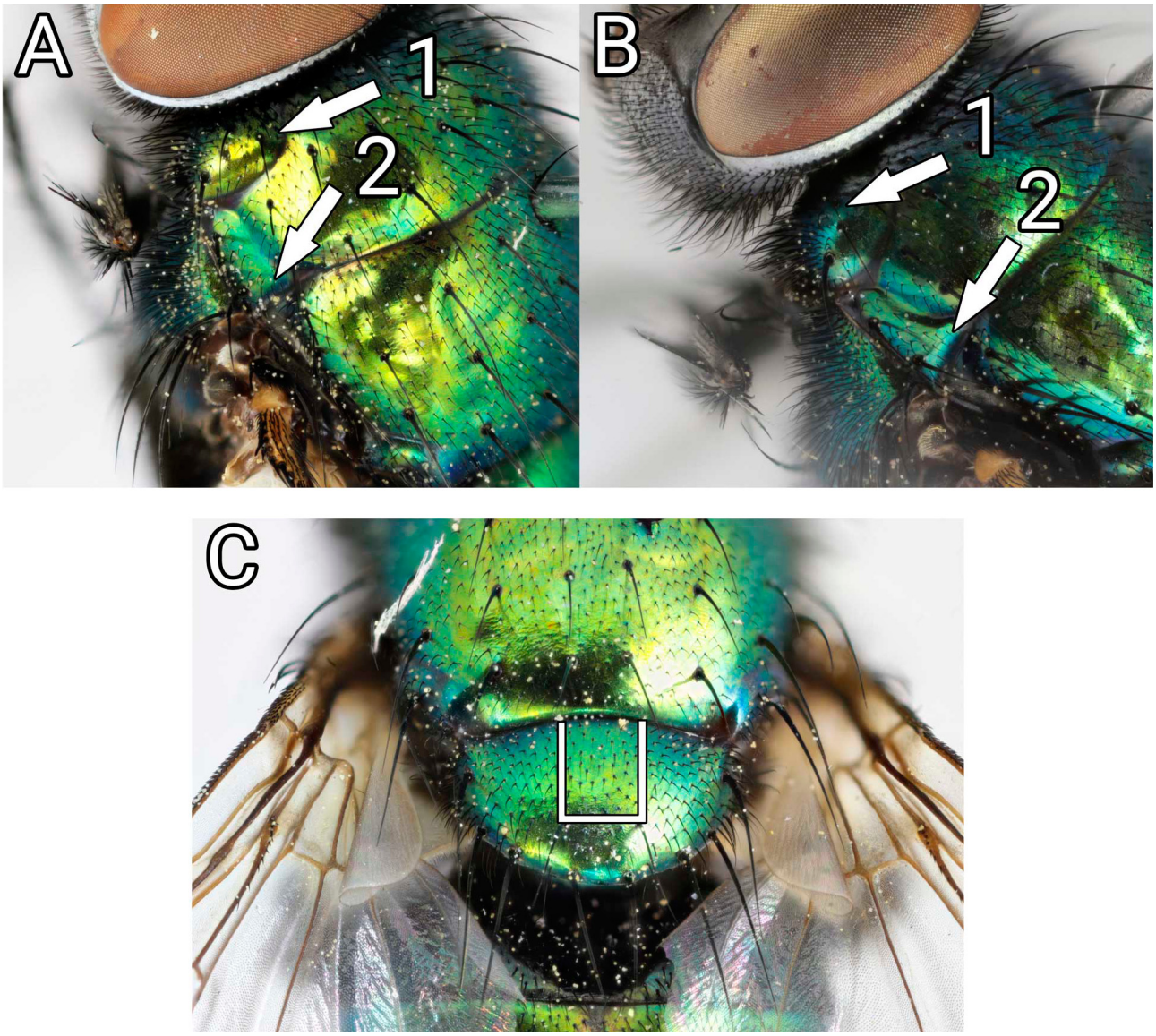

Fig. 2. Areas on the thorax of *Lucilia sericata* used in the analysis of traits of chaetotaxy: Posterior slope of humeral callus behind basal setae (1A, B), edge of notopleuron behind posterior notopleural seta (2A, B), "quadrat" between discal setae and anterior margin of scutellum (C).

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 callus 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*.

Locus	5'–3' sequence, Forward	5'–3' sequence, Reverse	Repeat motif
loc7	GGAAAAGGGGATGAAAGAGAGT	GTGAATCGTGTGTGGAAGTTTT	$(CA)_6$
loc17	TGCTCAGGATACAAACGTACA	GAATACAAAACCCTTAACCTCG	$(GT)_6$
loc19	TAGGAGCAGGAGAGAATGAA	TGACGTGGAATAATCATCAC	$(GT)_7$
L2	TGTCACCAAGAGTTGTAGAGCAA	ACGTTAGCGAATCAACCATGTA	$(CAA)_2$ CCA $(CAA)_2$ CCA $(CAA)_2$
AD1	GGTCCTACAGGGTTTTATTCA	TAGAGAGGTTTTGGTCACAGA	$(TC)_6$
AD6	CGAGTTTTATGTCTTCAGCC	CAGGAAATTAGGTCAGGAAAC	$(CA)_6$
AD8	TGTATCGTTCGGTCTGTAAGT	CTCAGTCCTCTACCAACTAAATG	$(TG)_7$
AD9	CCATTTTATTAGGCGTTCAG	CAACAAGAGTATCTGACAACCA	$(AC)_6$
AD10	GAATCTCAAAGCCTCCATAG	AAGTATTCAAGCCACAATCC	$(AC)_6$

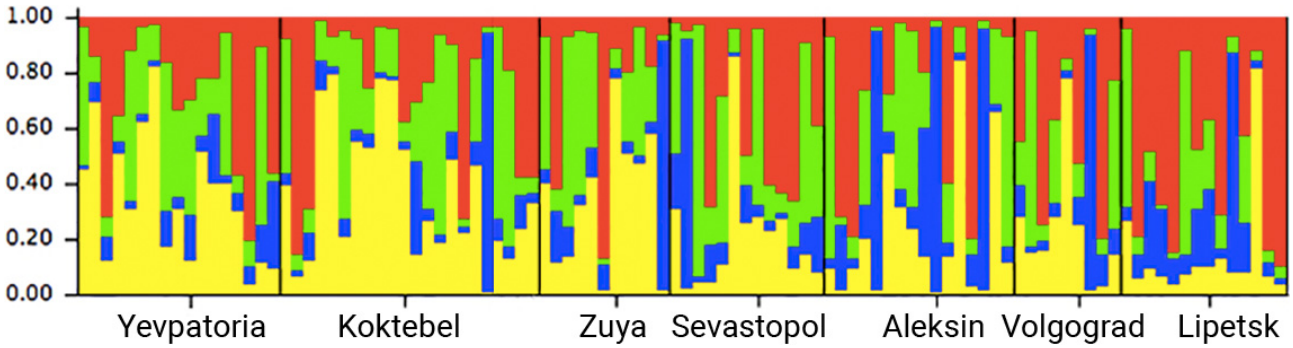

Fig. 3. STRUCTURE simulation of four clusters. Each vertical line represents the possibility of assigning a given specimen to one of the colour-coded clusters. Localities are indicated below the figure.

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). Pairwise comparisons were performed using the Tukey test (Fig. 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.

Table 3. Microsatellite loci analyzed in the genetic survey of *Lucilia sericata*.

Locus	Allele length (bp)	Number of alleles
loc7	150–162	7
loc17	243–284	20
loc19	206–236	4
L2	160–199	6
AD1	196–316	19
AD6	279–299	11
AD8	265–283	9
AD9	151–167	8
AD10	172–220	15

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 oviposit 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

Table 4. Two-way ANOVA of the distribution of chaetotaxy traits among samples of *Lucilia sericata* from different locations. "Location*Sex" is an interaction term, showing non-additive effect of the combination of two factors. *F* stands for the ratio of between-group variation to within-group variation. *P*-value is the probability of rejecting the true null hypothesis. Red colour indicates values sufficient for rejection.

	Number of setulae on "quadrat" between discal setae and anterior margin of scutellum		Number of hairs on posterior slope of humeral callus behind basal setae		Number of hairs on edge of notopleuron behind posterior notopleural seta	
	F	*P*	*F*	*P*	*F*	*P*
Location	1.659	0.163	0.889	0.496	1.549	0.193
Sex	5.859	0.019	3.996	0.051	2.773	0.102
Location*Sex	0.151	0.979	0.108	0.990	0.687	0.635

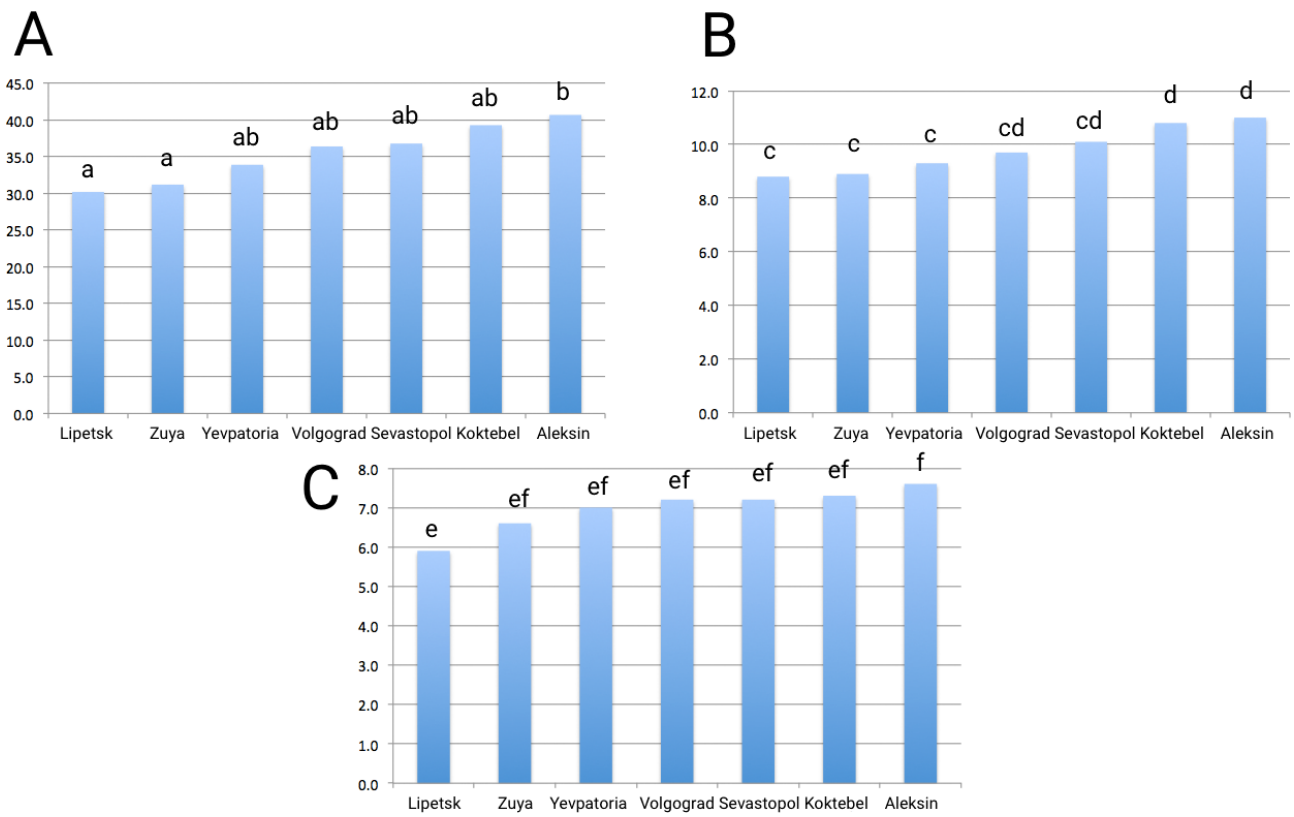

Fig. 4. Results of the Tukey test pairwise comparisons of the distribution of chaetotaxy traits among samples of *Lucilia sericata* from different locations. Y-axis shows number of setulae on "quadrat" between discal setae and anterior margin of scutellum for A, number of hairs on edge of notopleuron behind posterior notopleural seta for B, and number of hairs on posterior slope of humeral callus behind basal setae for C. Small letters above bars indicate statistical significance; the same letter indicates the absence of statistical difference between locations.

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.

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