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Genetic comparisons between North American and European populations of *Lumbricus terrestris* L.

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ABSTRACT

The common earthworm *Lumbricus terrestris* L. is an invasive species that was introduced to North America by European settlers. Subsequently, earthworms have been distributed by human activity, invaded a wide geographic range and changed previously earthworm-free ecosystems. In the present study we analyzed seven European and four North American populations from a wide geographic range at three formerly described nuclear microsatellite markers. All three markers produced multi-banding patterns and marker presence versus absence was scored in 88 narrow size intervals. Similar levels of genetic variation were observed for North American (Nei's gene diversity = 0.058, Shannon's $I = 0.100$) and European populations (Nei's gene diversity = 0.064, Shannon's $I = 0.104$). North American populations showed a higher similarity among each other than European populations in accordance with their recent introduction to North America. The relatively high level of genetic variation in North American populations and the high similarity among each other suggest their establishment from genetically diverse founder populations and rapid human-mediated population expansion. The source regions in Europe are still unclear from this analysis.

1. Introduction

Native North American earthworms have not fully recolonized North American forests since the last glaciation (Hendrix, 2006). The anecic earthworm, *Lumbricus terrestris* L. was first introduced to North America by early European settlers (Gates, 1976; Eisenhauer et al., 2007). Since then multiple introductions of *L. terrestris* to North America have occurred (Lindroth, 1957; Gates, 1976) and it has subsequently been distributed through human activities such as road constructions, transport of soils and release of unused fishing bait (Hendrix and Bohlen, 2002; Cameron et al., 2007). Today it is one of the most widely distributed exotic earthworms, having been reported in 38 US states and 10 Canadian provinces (Reynolds, 2008). *L. terrestris* is invasive over a wide geographic area, changing ecosystems that have evolved without earthworms (Bohlen et al., 2004; Hendrix, 2006; Scheu and Parkinson, 1994). Sequencing of the cytochrome oxidase I gene for nominal *L. terrestris*

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L. samples from Europe and North America revealed two species, *L. terrestris* and *Lumbricus herculeus*, in European samples, but only one species (*L. terrestris*) in North American specimen (James et al., 2010).

Earthworms are considered to be "ecosystem engineers" (Jones et al., 1994) because they modify the environment from its existing condition (Frelich et al., 2006; Lavelle et al., 2006). For example, anecic earthworms such as *L. terrestris* build permanent burrows, and pull large amounts of leaf litter into it, which they consume. They line their burrows with their casts, and deposit the rest of the cast on the soil surface (Zicsi et al., 2011) thus redistributing organic matter and minerals throughout the soil horizon. These alterations cause considerable changes in the composition and distribution of soil biota and ecosystem processes (Frelich et al., 2006; Szlavecz et al., 2011).

While the European origin of *L. terrestris* has been documented (Frelich et al., 2006; Hale et al., 2006), genetic studies comparing the level of genetic variation in potential source populations from Europe and North American populations are missing. Introductions from genetically diverse source populations are expected to maintain levels of genetic variation and thus the evolutionary potential ("invasiveness") of exotic species. Multiple introductions from genetically differentiated source populations can especially increase the level of genetic variation if formerly isolated populations come into secondary contact (e.g. Ellstrand and Schierenbeck, 2000; Kolbe et al., 2004). Genetic analyses can reveal the amount of genetic variation that exists within and among European and North American *L. terrestris* populations and provide insights into the geographic origin of North American earthworm populations and the importance of repeated introductions versus population expansion in the invasion of this species.

This project was designed to assess the population genetic structure of local North American and European earthworm populations. We hypothesize that North American earthworm populations (1) show no reduced genetic variation as compared to European populations due to their establishment from genetically diverse founder populations and subsequent population expansion, (2) are more similar to each other than European populations as result of a shorter divergence time, mixing via extensive transport, and the absence of severe genetic bottlenecks.

2. Material and methods

2.1. Sample collection

Earthworm samples were collected from 2004 to 2007 for seven European and for four North American regions in 2010 (Table 1). A varying number of individuals were sampled at each location by first identifying distinctive *L. terrestris* castings and burrows and then using different extraction techniques. Identification of earthworms was based on external characteristics (Schaefer, 1992). Sequencing of the cytochrome oxidase I gene (COI) in the European samples according to James et al. (2010) revealed the presence of only one species (Richter et al. in prep.). Electrical and chemical extraction, and hand sorting were performed for European populations, and mustard and formalin solutions were used for North American populations to extract earthworms from their subterranean burrows (Table 1). North American individuals located within the same sampling area were considered to belong to the same population and were taken from points spaced a minimum of 10 m apart along three straight line transects separated by 30 m. For European populations the sampling points were at least 5 m apart. All earthworms were transported within individual freezer bags supplied with leaf litter located within close proximity to the burrow from which they were taken. Upon collection, samples were stored in a cooler until returning to the lab where they were stored in a −80 °C freezer.

2.2. Sample preparation, DNA isolation, and polymerase chain reaction

Earthworm samples were individually cleaned with warm water and a 70% ethanol solution in preparation for tissue extraction. Small pieces of tissue located at the anterior end of the specimen were removed with a scalpel and forceps and used for DNA isolation. The reagents and methodologies outlined in the Qiagen DNeasy Blood and Tissue Kit were used for this process.

Table 1
Sampling locations.

Abbreviation	Location	Description	Latitude	Longitude	Sample year	Sampling method	*n*
BOS	Bosnia Herzegovina	Garden, Sarajevo	43.84N	18.36E	2006	Handsorting	24
FRA-Bru	France	Meadow, near Bruz	48.40N	1.45W	2006	Electrical	11
FRA-Béd	France	Meadow, near Bédee	48.12N	2.10W	2007	Chemical	12
FIN	Finland	Lawn, Jokioinen	60.81N	28.48E	2004	Handsorting	12
SWE	Sweden	Lawn, Uppsala	59.80N	17.65E	2004	Electrical	8
GER-Tim	Germany	Pasture, near Timmendorfer strand	53.96N	10.76E	2004	Handsorting	12
GER-Bay	Germany	Lawn, near Bayreuth	49.94N	11.72E	2004	Electrical	11
MI-Huron	Michigan	Forest, Huron Mountain reserve	46.89N	87.87W	2010	Mustard solution	21
MI-Houg	Michigan	Forest, Houghton	47.53N	93.47W	2010	Mustard solution	20
MD	Maryland	Forest edge, near Baltimore	39.34N	76.63W	2010	Formalin extraction	19
ME	Maine	Hemlock, sugar maple forest, Unity	44.60N	69.33W	2010	Mustard solution	9