Invasive lumbricid earthworms in North America—Different life histories but common dispersal?

Andreas Klein\(^1,2,3\) | Nico Eisenhauer\(^2,3\) | Ina Schaefer\(^1\)

\(^1\)Johann Friedrich Blumenbach Institute of Zoology and Anthropology, Animal Ecology, University of Göttingen, Göttingen, Germany
\(^2\)German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany
\(^3\)Institute for Biology, Leipzig University, Leipzig, Germany

Correspondence
Andreas Klein, Johann Friedrich Blumenbach Institute of Zoology and Anthropology, Animal Ecology, University of Göttingen, Göttingen, Germany.
Email: andreas.klein@biologie.uni-goettingen.de

Abstract

\textbf{Aim:} Lumbricid earthworms are invasive across northern North America, causing notable changes in forest ecosystems. During their range expansion, they encountered harsher climatic conditions compared to their native ranges in short time (~400 years). This study investigated if (a) dispersal barriers, (b) climatic selection or (c) anthropogenic activities, that is fishing bait disposal, structure the dispersal of free-living earthworm populations.

\textbf{Location:} North America, forest habitats along former Wisconsinan glaciation line.

\textbf{Taxon:} \textit{Lumbricus terrestris}, \textit{L. rubellus}.

\textbf{Methods:} \textit{Lumbricus terrestris} and \textit{L. rubellus} co-occur in the same habitats but differ in ecology and use as fishing bait. Both species were sampled in five transects ranging from the east to the west coast of northern North America, including major dispersal barriers, three different climate zones, and bait shops near sampling locations. Genetic diversity and structure were compared between the two species, and the presence of free-living bait shop genotypes was assessed using four markers (COI, 16S rDNA, 12S rDNA, and H3).

\textbf{Results:} Populations of both species were genetically diverse with some geographic structure, which was more pronounced in \textit{L. terrestris} than in \textit{L. rubellus}. Common haplotypes were present in all regions, but locally restricted haplotypes also occurred. Furthermore, two distinct genetic clades of \textit{L. terrestris} co-occurred only in the two most distant transects (Alberta and Minnesota). Genotypes identical to bait individuals were omnipresent in field populations of \textit{L. terrestris}.

\textbf{Main Conclusions:} Genetic diversity was high in both species, and invasive populations represented a genetic subset of European earthworms. Geographic and climatic dispersal barriers affected the less mobile species, \textit{L. terrestris}, resulting in differences in genetic structure between the two species. Our results indicate common long-distance dispersal vectors and vectors affecting only \textit{L. terrestris}. The roles of climate and anthropogenic activities are discussed, providing additional explanations of dispersal and new insights into establishment of invasive earthworm populations.

\textbf{Keywords} agriculture, biological invasion, climate, colonization, dispersal barriers, genetic clades

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2019 The Authors. \textit{Journal of Biogeography} Published by John Wiley & Sons Ltd.
1 | INTRODUCTION

European lumbricid earthworms are among the most successful invasive species in North America (James & Hendrix, 2004). European settlers introduced them at the east coast about 400 years ago, both accidentally and intentionally (Gates, 1976). Similar to many invasive species living above the ground, earthworms substantially alter the functioning of invaded ecosystems (Bohlen et al., 2004; Eisenhauer, Partsch, Parkinson, & Scheu, 2007; Hendrix et al., 2008; Mooney & Hobbs, 2000; Scheu & Parkinson, 1994). They change physical and biotic properties of the soil, which affects the density of other soil invertebrates, plant community composition, and aboveground food webs (Lee, 1985; Edwards & Bohlen, 1996; Eisenhauer et al., 2007; Eisenhauer 2010; Craven et al., 2017; Ferlian et al., 2018). In general, where earthworms are native they beneficially affect plant growth (van Groenigen et al., 2014) and plant competition (Eisenhauer & Scheu, 2008), but earthworms can exert contrasting effects on ecosystems that developed without their presence (Bohlen et al., 2004; Craven et al., 2017; Hale, Herms, Hansen, Clausen, & Arnold, 2005).

As successful invaders, earthworms possess high tolerance for a wide range of environmental conditions, though they prefer clay soils with near neutral pH that restricts their distribution (Curry, 2004; Fischelli, Freligh, Reich, & Eisenhauer, 2013; Laverack, 1961). Due to their ability to tolerate disturbances, they also occur in agricultural fields and meadows, with varying frequencies and abundances (Hendrix et al., 1992). In general, earthworms are susceptible to prolonged freezing periods, drought and geographic barriers like mountain ranges and large water bodies, which usually restrict their natural dispersal pattern (Eggleton, Inward, Smith, Jones, & Sherlock, 2009; Reynolds, 1994). However, they recently were recorded from interior Alaska and Fennoscandia suggesting that they can also withstand very low temperatures (Booysen, Sikes, Bowser, & Andrews, 2018; Wackett, Yoo, Olofsson, & Klaminder, 2018). Active dispersal of earthworms is slow, but they were able to spread across northern North America within a few hundred years by passive dispersal or repeated introductions, and today they are present in large areas from the east coast to the Midwest, east of the Rocky Mountains in Canada, and the Pacific coast (Hale et al., 2005; Holdsworth, Freligh, & Reich, 2007; Reynolds, 1977, 1994, 2016; Reynolds, Linden, & Hale, 2002; Scheu & Parkinson, 1994). The pronounced ecological consequences of earthworm invasions in North America are well documented, making earthworms one of the best-studied invasive animal species living below the ground (Wardle, Bardgett, Callaway, & Putten, 2011) and thus, a unique model system for biological invasion and accompanying effects (Hendrix et al., 2008).

During their expansion across northern North America, European earthworms established in distinct climate zones that differ in the amount and distribution of precipitation across the year, as well as frost intensity and duration, two abiotic factors that are known to drive earthworm distribution (Curry, 2004; Fischelli et al., 2013; Holmstrup, 2003; Uvarov, Tiunov, & Scheu, 2011). At the west coast, precipitation is high (1,200 mm/year), mild frost occurs sporadically and lasts for only a few weeks between December and January. By contrast, in the central plains of North America, precipitation is low (400–600 mm/year), and strong frost conditions typically persist between November and March, with occasional night frost already starting in late August and extending into early June. In the east, precipitation is intermediate (800–1,000 mm/year), and frost conditions typically last from December to February. Given this wide range of climatic conditions, knowledge on genetic diversity and relationships among populations across North America is needed for a better understanding of dispersal mechanisms and population establishment.

We investigated the genetic structure of *Lumbricus rubellus* and *L. terrestris*, two exotic earthworm species that are widespread and common across northern North America. Both feed on litter but have distinct ecological preferences and life histories (Sims & Gerard, 1999). *Lumbricus rubellus* is an epi-endogeic species, living in horizontal burrows up to 30-cm deep and moves freely within the litter layer for foraging. It prefers slightly acidic soils and generally has a higher pH and frost tolerance than *L. terrestris* (Addison, 2009; Tiunov, Hale, Holdsworth, & Vsevolodova-Perel, 2006). In contrast, *L. terrestris* prefers neutral to slightly alkaline soils, lives in permanent, vertical burrows of up to 2-m depth, and collects litter in the vicinity of its burrow entrance (Addison, 2009; Sims & Gerard, 1999; Tiunov et al., 2006). Active dispersal rates of the two earthworm species range between 2–4 m/year for *L. terrestris* and 10–14 m/year for *L. rubellus* (Marianissen & van den Bosch, 1992). As epi-endogeic species, passive dispersal of *L. rubellus* by human activities may be more likely by moving animals and cocoons in surface soils, such as through activities related to forestry or tourism (i.e., by hiking or vehicles). In contrast, *L. terrestris* lives in permanent, vertical burrows that are only left for foraging and mating (usually at night), making it less likely to be passively transported by human activities above the ground. *Lumbricus terrestris* is commonly used as fishing bait and sold in bait shops, which likely facilitates its dispersal. By contrast, *L. rubellus* is rarely sold in bait shops (A. Klein, pers. obs.). Disposal of fishing baits contributes substantially to the introduction and establishment of earthworm populations in recreational and fishing areas (Holdsworth et al., 2007; Keller et al., 2007), but the long-term establishment of these populations and further dispersal in the field remain unclear.

We sampled earthworms from five transects of ~150- to 300-km length (north-south orientation) in three climatic regions in two provinces in Canada and three states in the USA: the warm and moist region of British Columbia, Canada (BC), the cold and dry regions of Alberta, Canada (AL) and Minnesota, USA (MN), and the cold and moderately moist regions of Michigan, USA (MI) and New York State, USA (NY), respectively. This is the first study investigating the invasion of detritivorous soil animals on continental scale, including two different dispersal barriers and distinct climate zones in its sampling design.

We tested three hypotheses to understand if climate (H1), dispersal barriers (H2), and/or human migrations and transport (H3) predominantly structured the distribution and establishment of European earthworm species in northern North America: We tested, if (H1) distinct genetic clades from genetically diverse
source populations established in the different climate zones. Due to environmental filtering we expected to find monophyletic clades in the different regions, if only individuals survived that were better adapted to regional drought or cold conditions. Furthermore, the Rocky Mountains and the Great Plains may mark potential dispersal barriers for earthworms and we tested if (H2) earthworms were introduced independently in areas that are separated by potential dispersal barriers resulting in distinct genetic clades in the west (BC, AL), but less distinct clades or no genetic structure in the east (MN, MI, NY) where geographic dispersal barriers are of minor importance. Finally, we tested if human-mediated dispersal of earthworms counteracts local genetic structure and disregards dispersal barriers (H3), resulting in diverse earthworm populations and genotypes that are represented in all regions without any local clades occurring. To account for human-mediated dispersal by dumping of fishing baits, which is a severe problem in northern North America (Hale, 2008; Holdsworth et al., 2007; Seidl & Klepeis, 2011), we purchased earthworms from bait shops near sampling locations in all transects to test if bait genotypes contribute to free-living populations, thereby increasing local diversity.

2 | MATERIALS AND METHODS

2.1 | Sampling design—dispersal barriers and climate

Between May and July 2014 and in June 2015, we collected *L. terrestris* and *L. rubellus* along five transects (regions) spanning from east to west of the northern North American continent, ranging in the USA from New York State (Adirondack Mountains, transect NY), to the Midwest, that is Michigan (upper peninsula, transect MI) and Minnesota (near Minneapolis/St. Paul, transect MN; Table 1). In Canada, we collected earthworms east and west of the Rocky Mountains in Alberta (south of Calgary, transect AL) and British Columbia (near Vancouver, transect BC). Distances among transects ranged between 700 and 1,600 km, and within transects earthworms were collected at five sampling locations with north–south orientations.

| Sampling transect | Climate zone | Climate characteristics | Sampling location |
|-------------------|--------------|-------------------------|-------------------|
| British Columbia (BC) | Mixed Mediterranean, oceanic and continental | Warm and moist<br>AMP: ~1,200 mm/year<br>AMT: 6–16°C | Cypress Provincial Park<br>Golden Ears Provincial Park<br>Cultus Lake |
| Alberta (AL) | Cold continental | Cold and dry<br>AMP: 400–750 mm/year<br>AMT: −2 to 9°C | Crandell Lake<br>Waterton Springs<br>Maycroft<br>Eden Valley<br>Fish Creek Park, Calgary<br>Nose Hill Park, Calgary |
| Minnesota (MN) | Cold continental | Cold and dry<br>AMP: 400–750 mm/year<br>AMT: −2 to 9°C | Nerstrand<br>Wood-Rill SNA<br>Wolsfeld Wood SNA<br>Warner Nature Center<br>Pine Needles Preserve<br>Rush City |
| Michigan (MI) | Moderate continental | Cold and moderately moist<br>AMP: 800–1000 mm/year<br>AMT: 0–9°C | Turner<br>Tawas City<br>Alpena<br>Gaylord |
| New York (NY) | Moderate continental | Cold and moderately moist<br>AMP: 800–1000 mm/year<br>AMT: 0–9°C | Hamilton<br>Norwich<br>Newcomb<br>Lower Saranac Lake<br>Lake Placid<br>Portland Waterfront |

Note: See Appendix S1 Table S1 for GPS coordinates.
Abbreviations: AMP, annual mean temperature; AMT, annual mean temperature.
orientation that were 20–80-km apart. The two major dispersal barriers for plants and animals are the extensive dry grassland areas of the Great Plains extending between the transects of Minnesota (USA) and Alberta (Canada), and the Rocky Mountains, which separate the two Canadian transects Alberta and British Columbia. Climate in east and central northern North America is similar to continental climate in Europe, but seasonality in North America is harsher with hotter and drier summers, and longer and colder winters, which is most extreme in Alberta and Minnesota. Climate in British Columbia differs from that in Europe, as three different climate zones (Mediterranean, Continental and Oceanic) co-occur in the Greater Vancouver area.

Earthworms were collected in forests by turning over logs, hand sorting of litter, digging or applying mustard solution to extract earthworms from soil. Additionally, we purchased earthworms sold as fishing baits in bait shops close to sampling locations; all bait shops exclusively sold *L. terrestris*, restricting the bait shop dataset to a single species. Earthworms were washed, stored in 75% ethanol in the field and later transferred in the laboratory into 95% ethanol and stored at 16°C. One centimetre of tail tissue of each individual was cut and shipped to the University of Göttingen (Germany) for molecular analyses; remaining body parts are stored as voucher specimens at the University of Minnesota (Minneapolis-St. Paul, MN) and the University of British Columbia (Vancouver, BC).

### 2.2 | Genetic analyses

Genomic DNA was extracted with the Genaxxon DNA Tissue Mini Prep Kit (Genaxxon) following the manufacturer’s protocol. Four molecular markers were amplified: the mitochondrial genes COI (~600 bp; Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994), 16S rDNA (~750 bp; Pérez-Losada et al., 2009), and 12S rDNA (~400 bp; Simon et al., 1998), and the nuclear gene Histone 3 (~350 bp; Colgan et al., 1998). The PCR cycling conditions had an initial activation step at 95°C for 3 min, 40 amplification cycles (denaturation at 95°C for 30 s, annealing at 53°C for 60 s, elongation at 72°C for 60 s), and a final elongation step at 72°C for 10 min and were sequenced at the Göttingen Genome Sequencing Laboratory (Georg August University Göttingen) and SeqLab Göttingen (Microsynth). Sequences were submitted to the GenBank databases under the accession numbers (MK730610-MK731484) (GenBank: www.ncbi.nlm.nih.gov/genbank). Sequences were checked with Sequencher 4.9 (Gene Codes Corporation), and ambiguous positions were coded as wobble bases. Consensus sequences of the individual genes were assembled in BioEdit 7.0.1 (Hall, 1999) and aligned with ClustalW. Genes were analysed individually and in a combined matrix of 2.150 bp; all positions with wobble bases were deleted for further analyses. Sequence alignments (single genes and combined) were collapsed into haplotype alignments using FaBox 1.41 (Villesen, 2007). The best-fit models of sequence evolution were estimated with TOPALi v2.5 (Milne et al., 2004) using the Akaike information criterion (AIC; Akaike, 1973). Trees were constructed using MrBayes 3.2. (Ronquist et al., 2012), partitioning the combined alignment to

### 2.3 | Phylogeography and genetic differentiation across putative dispersal barriers

Spatial distribution of genetic clades was analysed with haplotype networks and constructed for 16S rDNA, which provided the most informative resolution. Median-joining (MJ) networks (Bandelt, Forster, & Röhl, 1999) were constructed with PopART (University of Otago, Dunedin, New Zealand) and edited using Inkscape (Software Freedom Conservancy). Parameters were set to equal weights for all mutations and the epsilon parameter to zero to restrict the choice of possible links in the final network.

To test hypotheses about climatic and geographic dispersal barriers, we used analyses of molecular variance (AMOVA) and analysed genetic differentiation among populations using the distance method of Tajima & Nei, pairwise differences without Gamma correction, and pairwise genetic distances using Arlequin 3.5.2.2 (Excoffier et al., 2005). AMOVAs were calculated with COI, the most variable gene regarding nucleotide diversity (Appendix S2 Table S5), and earthworm populations were assigned a priori according to our first hypotheses (H1) into climate zones separating populations from British Columbia (mixed climate), Alberta and Minnesota (cold continental climate), Michigan and New York (moderate continental climate). To test for the relevance of geographic barriers (H2), populations were analysed in three different combinations: Great Plains as main dispersal barrier (BC, AL vs. MN, MI, NY), Rocky Mountains as main dispersal barrier (BC vs. AL, MN, MI, NY) and Great Plains and Rocky Mountains as main dispersal barriers (BC vs. AL vs. MN, MI, NY). Human influence on reducing the effect of dispersal barriers was tested by comparing genetic variance among transects (BC vs. AL vs. MN vs. MI vs. NY). If human transport plays a significant role for earthworms across large geographic distances (H3), genetic variance should be similar among regions.

### 2.4 | Climate data

The responses of genetically diverse earthworms to ecological factors were inspected using a multiple regression matrix (MRM). Bioclimatic data were retrieved from WorldClim v2 bioclimatic variables database (Fick & Hijmans, 2017) and had a spatial resolution of ~5 km². The response matrix compared genetic pairwise differences
of the COI sequence data and was calculated with the Analysis of Phylogenetics and Evolution (ape) package (Paradis, Claude, & Strimmer, 2004) in R (http://www.R-project.org). Tested factors were (a) environmental abiotic parameters, that is, annual mean temperature (BIO01), maximum temperature of the warmest month (BIO05), minimum temperature of the coldest month (BIO06), mean temperature of the wettest quarter (BIO08), mean temperature of the driest quarter (BIO09), annual precipitation (BIO12), precipitation of the driest month (BIO14), precipitation seasonality (BIO15), and (b) the geographical parameter spatial distance and elevation. Data were first transformed into scaled explanatory distance matrices using Euclidean distances for standardization and then normalized. Information on the correlation of the environmental variables is provided in Appendix S2 (Table S6). With the present dataset from the sampling locations provided in Table 1, we have limited power to clearly separate the different climatic drivers that are also likely to interactively drive earthworm communities. As a consequence, our results included several correlated climate variables and should be interpreted with caution, but inspire future work on the clear identification of different environmental drivers and their interactions in affecting earthworm distribution patterns. The spatial distance between each pair of samples was calculated using the Geographic Distance Generator v1.2.3 (Ersts, 2014) with the World Geodetic System (1984) setting for the reference spheroid and then normalized by dividing the values by the maximum distance value, thus measuring the absolute but normalized distances. The MRM function was executed with the R package ecodist (Goslee & Urban, 2007).

We also examined if additional environmental factors (i.e. sampling location, soil pH and human population density) correlated with earthworm abundance and genetic diversity (see Appendix S2 Figures S2–S3). Results indicated a trend that human densities affect genetic diversity and abundances of L. terrestris but not of L. rubellus.

3 | RESULTS

3.1 | Sampling and genetic diversity

In total, 120 L. rubellus (LR) and 122 L. terrestris (LT) individuals were sampled from the 25 locations. The number of individuals per transect varied from 12 to 48 for L. terrestris and from 12 to 37 for L. rubellus (Appendix S1 Figure S1). Nucleotide (NUD) and haplotype diversity (HTD) was greater in L. rubellus and decreased in both species from COI to 16S rDNA to 12S rDNA to H3. Overall, nucleotide diversity of COI was two or three times higher in L. rubellus than in L. terrestris and varied among transects (Appendix S1 Tables S2, S3).

![Figure 1](image-url)
Relatedness and spatial distribution

In both species, earthworms were closely related resulting in phylogenetic trees with a weakly supported backbone and clades with mixed geographic origin. Accordingly, phylogenetic and geographic structure was generally weak, in particular in *L. rubellus*. However, in both species, some populations formed well-supported clades (posterior probabilities: 0.95-1; Figure 1a) that were also recovered by haplotype network analyses. In *L. rubellus*, two clades comprised closely related individuals from all transects (mixed clades 1 and 4 with 37 and 60 individuals, respectively). However, five individuals from Minnesota (clade 2, green) and 18 individuals from New York (clade 3, blue) were distinct and did not occur in other transects (Figure 1b). All North American COI haplotypes of *L. rubellus* could be assigned to lineages from Europe (Giska, Sechi, & Babik, 2015; Sechi, 2013). Haplotypes in clade 4 corresponded to the widespread European lineages A1-A3. Haplotypes in clade 1 and 2 clustered with European lineages C and D from Eastern Europe (Poland, Hungary, Balkans), and haplotypes in clade 3 clustered with lineage H, which is restricted to Germany and Austria. We compared COI lineages with the H3 dataset to check if mitochondrial and nuclear markers corresponded. The North American haplotypes of the COI clades 1, 2 and 4 carried the same H3 lineage, which is undescribed in Europe. Clade 3 comprised several H3 haplotypes, one known from Europe (Martinsson & Erséus, 2017) and one also present as widespread H3 lineage in the common COI clade 4.

Genetic distances among populations of *L. terrestris* were less distinct but had more haplotypes separating into more clades than *L. rubellus* (Figure 2a,b). The largest clade of *L. terrestris* (clade 2, 52 individuals) included haplotypes from all transects. The second largest clade (clade 1, 32 individuals) consisted of a haplotype predominantly found in Alberta (orange) and Minnesota (green) and in one individual from New York (blue). Furthermore, haplotypes from Alberta also occurred in separate clades together with Minnesota (clade 4, 7 individuals), British Columbia (red, single individual) and Michigan (violet, clade 5, 16 individuals). Notably, Minnesota and British Columbia also had distinct haplotypes that formed isolated monophyletic clades (clades 6, 5 individuals and 3, 5 individuals).

Most haplotypes of *L. terrestris* from bait shops were identical to common and widespread haplotypes from field populations (Figure 3). Only few haplotypes formed separate clades (mainly AL and BC) or were related to rare field haplotypes (BC) from the same sampling region. The North American COI and H3 haplotypes of *L. terrestris* were closely related or identical to haplotypes described from Europe or North America in previous studies (Appendix S1 Table S4, Appendix S3 Figures S4–S5).
3.3 | Genetic differentiation across putative barriers

Analysis of molecular variance (AMOVA) across all four genes showed that most of the molecular variance was at local scale (within sampling points = populations, Appendix S2 Table S5), with ~92%–94% of variance in *L. rubellus* and ~70% to 73% in *L. terrestris* in the most variable gene (COI, Table 2). In both species, molecular variance predominantly resided at population level but was much clearer in *L. rubellus* with only 3.75% of variance among populations compared to *L. terrestris* with 17.92% (Table 2). Analyses based on a priori assigned populations to test for effects of climate (H1: transects BC vs. AL, MN vs. MI, NY), geographic barriers (H2: Great Plains = transects BC, AL vs. MN, MI, NY; Rocky Mountains = transects BC vs. AL, MN, MI, NY; Great Plains and Rocky Mountains = transects BC vs. AL vs. MN, MI, NY), and distance (H3: transects BC vs. AL vs. MN vs. MI vs. NY) on population structure also showed very little variance for *L. rubellus* within (3.75%–5.81%) and among geographic populations (0.71%–2.86%), thereby rejecting all hypotheses for this species. However, *L. terrestris* generally showed a higher genetic structure with 11.22% variance among climate regions (H1) followed by distance among regions (9.46%).

3.4 | Importance of bioclimatic factors

The MRM showed contrasting results for the two earthworm species; the permutation test indicated that 22% and 4% of the variance were explained by climatic variables for the complete datasets of *L. rubellus* and *L. terrestris*, respectively (Appendix S2 Table S7). *Lumbricus rubellus* correlated significantly (*p* < 0.002) with all tested bioclimatic factors except for the minimum temperature in the coldest month (BIO06; *p* = 0.755) and seasonality of precipitation (BIO15; *p* = 0.084). For the complete dataset of *L. terrestris*, correlations generally were not significant (*p* > 0.130), except for the minimum temperature of the coldest month (BIO06; *p* = 0.022). We repeated the analysis with reduced datasets containing only the local clades (clades 2 and 3 of *L. rubellus*, all clades except the widespread clade 2 of *L. terrestris*) and the widespread clades (clades 1 and 4 of *L. rubellus*, clad 2 of *L. terrestris*). In the reduced datasets, the variance explained by climatic factors decreased strongly for *L. rubellus*, but increased for the local clades of *L. terrestris* (Table 3). Temperature and seasonal precipitation explained 22% (*r^2^ = 0.22, *p* = 0.001) of the variance of the local clades of *L. terrestris*, correlations for the widespread clade of *L. terrestris* were not significant.
DISCUSSION

4.1 Genetic diversity

This study shows that northern North American populations of the two earthworm species *L. rubellus* and *L. terrestris* share the same genetic lineages with populations of their native range in Europe. However, genetic diversity is lower in North America than in Europe, which is typical for invasive species (Sakai et al., 2001; Allendorf & Lundquist, 2003; King, Tibble, & Symondson, 2008; Gailing et al., 2012; Donnelly et al., 2013; Donnelly, Harper, Morgan, Pinto-Juma, & Bruford, 2014; Giska et al., 2015). Consistent with studies in Europe, genetic diversity in *L. rubellus* was higher than in *L. terrestris* (King et al., 2008; Martinsson & Erséus, 2017), and intraspecific genetic distances of COI were comparable with those reported from Europe (King et al., 2008; James et al., 2010; Klarica, Kloss-Brandstätter, Traugott, & Juen, 2011).

In North America, common and widespread haplotypes dominated in both species, but genetic and geographic structure differed. Among populations of *L. rubellus*, haplotypes divided into two genetic lineages that predominantly occurred in all sampling regions (except in New York), which belong to common and widespread lineages in Europe. Human mediated long-range dispersal by passive transport is more likely for *L. rubellus*, which frequently moves in leaf litter near or on the soil surface than for soil-dwelling anecic species. However, dispersal via bait abandonment in the past cannot be excluded. In contrast, *L. terrestris* was more likely to occur in bait shops in North America today, and a large fraction of individuals from bait shops we purchased earthworms from.
spread of *L. terrestris*. Historically, earthworms sold as fishing baits were collected from fields and sold locally, but establishment of refrigerated warehouses by large distributors selling pre-packed baits nationwide might additionally contribute to long-distance spread of genetic diversity. This assumption is supported by a study at local scale in Calgary, Alberta, that demonstrated the genetic relatedness of bait and field populations with fine resolution markers (Klein et al., 2017). Here, at large scale, bait haplotypes from Alberta and British Columbia in part did not match the haplotypes of nearby field populations, but rather field populations of far distant transects. However, bait cannot be the only source and vector for dispersal of *L. terrestris*, since the two most distant transects of Alberta and Minnesota contained three genetic clades that occurred nowhere else, indicating the existence of a distinct dispersal vector that connects these two transects.

### 4.2 Climate and dispersal barriers

Genetic variation among regions was very low for *L. rubellus*, and bioclimatic factors or dispersal barriers did not explain the distribution of common lineages, which agrees with its higher tolerance to frost (Fisichelli et al., 2013; Sims & Gerard, 1999; Tiunov et al., 2006). The ability of epi-endogeic earthworms to quickly adapt to cold and fluctuating temperatures through behavioural and physiological changes (Holmstrup, 2003), and their persistence to perturbations, such as heavy metal pollution by fertilizers and intoxication by pesticides, are well known (Edwards & Bohlen, 1996; Kruse & Barrett, 1985; Levine, Hall, Barrett, & Taylor, 1989). Although consecutive summer droughts can have strong effects on epi-endogeic earthworms, drought resistance of cocoons allows persistence through dry periods (Eggleton et al., 2009; Holmstrup & Loeschcke, 2003).

In contrast to *L. rubellus*, genetic variance in the common lineages of *L. terrestris* in part was related to climate factors, in particular frost, drought and seasonality. These results corresponded to findings that anecic earthworm species are negatively affected by prolonged drought periods, high frequency of freeze-thaw cycles and low soil moisture during their prime reproductive periods in spring and autumn (Addison, 2009; Curry, 2004; Sims & Gerard, 1999). Conform to these findings, the distinct genetic composition of populations in Alberta and Minnesota correlated with the continental climate in both transects. However, both species were recorded from areas with harsh frost conditions (Booysen et al., 2018; Wackett et al., 2018). These areas were associated with recent human introductions and human land use indicating potential new stepping stones of earthworm invasions. If the more severe frost and drought periods in these regions facilitated genetic diversity by continuous extinctions and reintroductions, or if only climatically pre-adapted lineages were able to establish viable populations in these areas needs to be investigated under controlled experimental conditions (Holmstrup, 2003). Additionally, joined analyses of more molecular datasets from North America and Europe would be an important step to explore the historical and recent invasion routes of exotic earthworms. Moreover, the application of more variable markers, for example, SNPs or microsatellites, would provide a more detailed picture on gene flow.
from which we can infer dispersal barriers and migration routes or the extent of genetic bottlenecks earthworms experienced during their invasion.

5 | CONCLUSIONS

Genetic diversity and structure of the two invasive earthworm species *L. rubellus* and *L. terrestris* was homogenous across all regions indicating a dominant common dispersal vector and the ability to adjust to most environmental conditions in northern North America. However, *L. terrestris* was genetically more structured, and here its genetic variance positively correlated with harsh climatic conditions in central North America. In contrast to *L. rubellus*, this species is common in arable fields with frequent disturbances, and distinctness of genetic lineages occurring predominantly in transects of Alberta and Minnesota could be explained by their position at the edges of the North American corn belt. Genetic patterns indicate that both species have common long-distance distribution vector(s). For *L. terrestris*, nation-wide bait distributors potentially play a major role as dispersal agent of field populations. In the past two decades, the globalization of economy has changed infrastructure, intensity and range of traffic including commercial distribution of soil-related goods, and potentially will increase dispersal of *L. rubellus* and *L. terrestris*.

Our present study exemplifies how earthworms as belowground invaders with substantial differences in life history traits can be used to test broad questions in invasion ecology, such as the genetic underpinnings of successful invasion events, geographic and climatic dispersal barriers, as well as the human role in ecologically relevant invasions. Thus, the present results may inspire future work on the role of different hypothesized main drivers of invasive species that can be explored with comparative genetic analyses (Sovic, Carstens, & Gibbs, 2016).

ACKNOWLEDGEMENTS

We gratefully acknowledge the following people. For collections: Timothy McCay (Colgate University) and Alex Roth (University of Minnesota); for logistical support and field assistance: Erin Cameron (University of Alberta), Cindy Buschena (University of Minnesota), Alice Chang (University of British Columbia), Zoe Jeffrey, Stacy McNulty (SUNY-ESF), Bastian Heimburger and Simon Dopichay (University Göttingen). Permission to collect specimens was provided by the Department of Natural Resources Minnesota, the Warner Nature Center and the Waterton Lakes National Park of Canada. Furthermore, we like to thank Stefan Scheu (University Göttingen) for his support and insightful discussions. This project was supported by the German Research Foundation (EI B62/7-1, SCHA1671/5-1, and DFG FZT 118) and the European Research Council (ERC Starting Grant 677232 to NE, ECOWORM).

REFERENCES

Addison, J. A. (2009). Distribution and impacts of invasive earthworms in Canadian forest ecosystems. *Biological Invasions*, 11, 59–79. https://doi.org/10.1007/s10530-008-9320-4

Akaike, H. (1973). Information theory and an extension of the maximum likelihood principle. In B. N. Petrov, & F. Csaki (Eds.), *Proceedings of the 2nd International Symposium on Information Theory* (pp. 267–281). Budapest, Hungary: Akademiai Kiado.

Allendorf, F. W., & Lundquist, L. L. (2003). Introduction: Population biology, evolution, and control of invasive species. *Conservation Biology*, 17, 24–30. https://doi.org/10.1046/j.1523-1739.2003.02365.x

Bandelt, H. J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, 16, 37–48. https://doi.org/10.1093/molbev/16.1.37

Bohlen, P. J., Scheu, S., Hale, C. M., McLean, M. A., Migge, S., Groffman, P. M., & Parkinson, D. (2004). Non-native invasive earthworms as agents of change in northern temperate forests. *Frontiers in Ecology and the Environment*, 2, 427–435. https://doi.org/10.1890/1540-9295(2004)002[0427:NIEAAO]2.0.CO;2

Booysen, M., Sikes, D., Bowser, M. L., & Andrews, R. (2018). Earthworms (Oligochaeta: Lumbricidae) of Interior Alaska. *Biodiversity Data Journal*, 6, 13. https://doi.org/10.3897/BDJ.6.e27427

Cameron, E. K., Bayne, E. M., & Colman, D. W. (2008). Genetic structure of invasive earthworms *Dendrobaena octaedra* in the boreal forest of Alberta: Insights into introduction mechanisms. *Molecular Ecology*, 17, 1189–1197. doi.org/10.1111/j.1365-294X.2007.03603.x

Cameron, E. K., & Bayne, E. M. (2009). Roadage and its importance in earthworm invasion of northern boreal forests. *Journal of Applied Ecology*, 46, 28–36. https://doi.org/10.1111/j.1365-2664.2008.01535.x

Casson, J., Shackleford, I., Parker, L., & Schultz, J. (2002). Conservation approach for goblin fern, *Botrychium mormo* W.H. Wagner. USDA Forest Service, Eastern Region, Milwaukee, WI. Unpublished report. Retrieved from http://www.fs.fed.us/r9/wildlife/tes/ca-overview/docs/Goblin-Fern-Approach_0602.pdf, pp. 4, 11, 19.

Colgan, D. J., McLaughlin, A., Wilson, G. D. F., Livingston, S. P., Edgecombe, G. D., Macaranas, J., & Gray, M. R. V. (1998). Histone H3 and U2 snRNA DNA sequences and arthropod evolution. *Australian Journal of Zoology*, 46, 419–437.

Craven, D., Thakur, M. P., Cameron, E. K., Frellich, L. E., Beauséjour, R., Blair, R. B., Eisenhauer, N. (2017). The unseen invaders: Introduced earthworms as drivers of change in plant communities in North American forests (a meta-analysis). *Global Change Biology*, 23, 1065–1074. https://doi.org/10.1111/gcb.13446

Curry, J. P. (2004). Factors affecting the abundance of earthworms in soils. In C. A. Edwards (Ed.), *Earthworm ecology* (2nd ed.), Chapter 6. Boca Raton, FL: CRC Press LLC.

Donnelly, R. K., Harper, G. L., Morgan, A. J., Orozco-Terwengel, P., Pinto-Juma, G. A., & Bruford, M. W. (2013). Nuclear DNA recapitulates the cryptic mitochondrial lineages of *Lumbricus rubellus* and suggests the existence of cryptic species in an ecotoxological soil sentinel. *Biological Journal of the Linnean Society*, 110, 780–795.

Donnelly, R. K., Harper, G. L., Morgan, A. J., Pinto-Juma, G. A., & Bruford, M. W. (2014). Mitochondrial DNA and morphological variation in the sentinel earthworm species *Lumbricus terrestrial*. 

**ORCID**

Andreas Klein https://orcid.org/0000-0002-3259-9973

Nico Eisenhauer https://orcid.org/0000-0002-0371-6720

Ina Schaefer https://orcid.org/0000-0002-2841-4219
Lee, K. E. (1985). Earthworms, their ecology and relationships with soils and land use. Sydney, Australia: Academic Press.

Levine, M. B., Hall, A. T., Barrett, G. W., & Taylor, D. H. (1989). Heavy metal concentrations during ten years of sludge treatment to an old‐field. Journal of Environmental Quality, 18, 411–418. https://doi.org/10.2134/jeq1989.00472422001800180040003x

Martinson, S., & Erséus, C. (2017). Cryptic speciation and limited hybridization within lumbricus earthworms (Citellata: Lumbricidae). Molecular Phylogenetics and Evolution, 106, 18–27. https://doi.org/10.1016/j.ympev.2016.09.011

Milne, I., Wright, F., Marshall, D. F., Husmeier, D., & McGuire, G. (2004). TOPALI: Software for automatic identification of recombinant sequences within DNA multiple alignments. Bioinformatics, 20, 1806–1807. https://doi.org/10.1093/bioinformatics/bth155

Mooney, H. A., & Hobbs, R. J. (2000). Invasive species in a changing world. Washington, DC: Island Press.

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R Language. Bioinformatics, 20, 289–290. https://doi.org/10.1093/bioinformatics/btg412

Pérez‐Losada, M., Ricoy, M., Marshall, J. C., & Domínguez, J. (2009). Phylogenetic assessment of the earthworm Aporrectodea caliginosa species complex (Oligochaeta: Lumbricidae) based on mitochondrial and nuclear DNA sequences. Molecular Phylogenetics and Evolution, 52, 293–302. https://doi.org/10.1016/j.ympev.2009.04.003

Reynolds, J. W. (1977). The earthworms (Lumbricidae and Sparganophilidae) of Ontario. Toronto, Canada: Life Science, Royal Ontario Museum.

Reynolds, J. W. (1994). The distribution of the earthworms (Oligochaeta) of Indiana: A case for the post quaternary introduction theory for megadriile migration in North America. Megadrilogica, 5, 13–22.

Reynolds, J. W., Linden, D. R., & Hale, C. M. (2002). The earthworms of Minnesota (Oligochaeta: Acanthodrilidae, Lumbricidae and Megascolecida). Megadrilogica, 8, 85–100.

Reynolds, J. W. (2016). Earthworms (Oligochaeta: Lumbricidae and Megascolecidae) in the northern Minnesota wetlands ecoregion (49), USA. Megadrilogica, 20, 74–78.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., … Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61, 539–542. https://doi.org/10.1093/sysbio/sys029

Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., … Wellar, S. G. (2001). The population biology of invasive species. Annual Review of Ecology, Evolution and Systematics, 32, 305–332. https://doi.org/10.1146/annurev.ecolsys.32.081501.114037

Sechi, P. (2013). An evolutionarily history of the peregrine epigeic earthworm Lumbricus rubellus. Cardiff, UK: Cardiff University.

Scheu, S., & Parkinson, D. (1994). Effects of earthworms on nutrient dynamics, carbon turnover and microorganisms in soils from cool temperate forests of the Canadian Rocky Mountains: a laboratory study. Applied Soil Ecology, 1, 113–125. https://doi.org/10.1006/appo.1994.0001-0

Seidl, D. E., & Klepeis, P. (2011). Human dimensions of earthworm invasion in the Adirondack State Park. Human Ecology, 39, 641–655. https://doi.org/10.1007/s10745-011-9422-y

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., & Flok, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America, 87, 651–701. https://doi.org/10.1093/aesa/87.6.651

Sims, R. W., & Gerard, B. M. (1999). Earthworms—Synopses of the British Fauna, No. 31. Dorchester, Great Britain: The Dorset Press.

Sovic, M. G., Carstens, B. C., & Gibbs, H. L. (2016). Genetic diversity in migratory bats: Results from RADseq data for three tree bat species at an Ohio windfarm. PeerJ, 4, e1647. https://doi.org/10.7717/peerj.1647

Terhivuo, J., & Saura, A. (1997). Island biogeography of North European parthenogenetic Lumbricidae: I. Clone pool affinities and morphometric differentiation of Åland populations. Ecography, 20, 185–196. https://doi.org/10.1111/j.1600-0587.1997.tb00361.x

Tiunov, A. V., Hale, C. M., Holdsworth, A. R., & Vsevolodova-Perel, T. S. (2006). Invasion patterns of Lumbricidae into the previously earthworm-free areas of northeastern Europe and the western Great Lakes region of North America. Biological Invasion, 8, 1223–1234. https://doi.org/10.1007/s10530-006-9018-4

Uvarov, A. V., Tiunov, A. V., & Scheu, S. (2011). Effects of seasonal and diurnal temperature fluctuations on population dynamics of two epigeic earthworm species in forest soil. Soil Biology and Biochemistry, 43, 559–570. https://doi.org/10.1016/j.soilbio.2010.11.023

Van Groenigen, J. W., Lubbers, I. M., Vos, H. M. J., Brown, G. G., De Deyn, G. B., & van Groenigen, K. J. (2014). Earthworms increase plant production: A meta-analysis. Scientific Reports, 4, 6365.

Villesen, P. (2007). FaBox: An online toolbox for FASTA sequences. Molecular Ecology Resources, 7, 965–968. https://doi.org/10.1111/j.1471-8286.2007.01821.x

Wackett, A. A., Yoo, K., Olofsson, J., & Klaånder, J. (2018). Human-mediated introduction of geoengineering earthworms in the Fennoscandinavian arctic. Biological Invasions, 20, 1377–1386. https://doi.org/10.1007/s10530-017-1642-7

Wardle, D. A., Bardgett, R. D., Callaway, R. M., & Van der Putten, W. H. (2011). Terrestrial ecosystem responses to species gains and losses. Science, 332, 1273–1277. https://doi.org/10.1126/science.1197479

BIOSKETCH

Andreas Klein is interested in phylogeography, invasion biology and population genetics of lumbricid earthworms, with particular interest in dispersal vectors and climate adaptation. This study is part of his PhD work at the universities of Göttingen and Leipzig on the spread of European earthworms in North America.

Author contributions: A.K., N.E. and I.S. conceived the original idea. A.K. conducted the field work, collected and analysed the data, and wrote the manuscript. All authors contributed to this study and the manuscript in form of discussions, suggestions and revisions, and approved the final manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Klein A, Eisenhauer N, Schaefer I. Invasive lumbricid earthworms in North America—Different life histories but common dispersal? J Biogeogr. 2020;47:674–685. https://doi.org/10.1111/jbi.13744