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ORIGINAL ARTICLE

Colonization history of *Metrioptera roeselii* in northern Europe indicates human-mediated dispersal

Peter Kaňuch[1,2]*, Åsa Berggren[1] and Anna Cassel-Lundhagen[1]

[1]Department of Ecology, Swedish University of Agricultural Sciences, 750 07, Uppsala, Sweden, [2]Institute of Forest Ecology, Slovak Academy of Sciences, 960 53, Zvolen, Slovakia

ABSTRACT

Aim The bush-cricket *Metrioptera roeselii* is an example of an insect which has expanded its indigenous range beyond expectations based on its natural dispersal potential. Understanding how species colonize new areas is vital for formulating effective species conservation programmes and managing invasive species. The aim of this research is to use mitochondrial sequence and microsatellite data to delineate the likely origin and dispersal pathways of *M. roeselii* in northern Europe. The well-known ecology of the species and the detailed colonization data make it a very suitable model species for addressing questions relating to invasiveness.

Location Fennoscandia, Baltic Sea coast, northern Europe.

Methods Using a 676 bp fragment of the mitochondrial cytochrome oxidase subunit I (*COI*) gene and seven polymorphic microsatellite loci, we genotyped and compared populations at 28 sites within the continuous range of *M. roeselii* along the Baltic Sea coast, and 10 isolated populations in Denmark, islands in the Baltic Sea and the Scandinavian Peninsula. The acquired data, information on the species' ecology and historical population establishment records were used to infer the colonization history and pathways of this species.

Results Both mitochondrial DNA and microsatellite data indicated that several of the isolated populations did not originate from their nearest locations within the continuous distribution area of *M. roeselii*. Instead, the likeliest source populations were in some cases situated > 500 km from the isolated populations. Hence the first records of appearance in the isolated sites did not coincide with the species' natural expansion but agreed well with the time of colonization of the founder sites inferred from the genetic data.

Main conclusions The limited ability of *M. roeselii* to cross geographical barriers through active dispersal, the inferred colonization pathways from this study, and the knowledge that transport of eggs can potentially occur with agricultural products collectively suggest that at least some of the isolated populations originate from human-mediated introductions rather than natural dispersal.

Keywords

COI gene, colonization history, dispersal, microsatellites, mtDNA, Orthoptera, range expansion, Roesel's bush cricket.

*Correspondence: Peter Kaňuch, Institute of Forest Ecology, Slovak Academy of Sciences, Ľ. Štúra 2, 960 53 Zvolen, Slovakia.
E-mail: kanuch@netopiere.sk

INTRODUCTION

The expansion of a species into previously unoccupied areas may be mediated through active dispersal by the species or other, passive mechanisms. Air or water currents may cause a natural passive transport of individuals far beyond their natural range (e.g. Gangwere & Llorente, 1992; Peck, 1996; Ashmole & Ashmole, 1997; Gatehouse, 1997), but new establishments are often also caused by human activity (e.g. Whinam *et al.*, 2005; Wilson *et al.*, 2009). The number of non-native species introductions has increased dramatically in recent decades in association with rapid increases in human-mediated travel and transport of various goods, especially agricultural products (di Castri, 1989).

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Genetic tools are very useful for tracing the origins of populations (Muirhead *et al.*, 2008; Estoup & Guillemaud, 2010). Identifying sources of introduced populations is important for both biodiversity conservation and pest management (Cox, 2004; Kenis *et al.*, 2009), as reconstructing colonization pathways is essential for controlling and preventing future invasions (Wilson *et al.*, 2009). In addition, such information is fundamental when studying evolutionary processes related to population expansion (Keller & Taylor, 2008). Knowledge of the biological traits of species is also important for understanding the evolutionary processes that determine colonization success, although it is unclear whether rapid adaptations of quantitative traits allow expansion, coincide with expansion, or are consequences of expansion (see Lee, 2002; Keller & Taylor, 2008; Whitney & Gabler, 2008; Estoup & Guillemaud, 2010).

A reliable discrimination of the factors behind long-distance passive transport, i.e. between natural processes or human activities, is difficult as dispersal events are random and hard to predict (Wilson *et al.*, 2009). Geographically, the Baltic Sea constitutes a natural barrier to the spread of species in northern Europe (Tyler, 2000; Cassel & Tammaru, 2003; Besold *et al.*, 2008) because it separates the Scandinavian peninsula and various islands from continental Europe. This area has a long history of trade and transport of diverse goods among countries surrounding the Baltic Sea, and extensive cargo traffic across it. Thus, possibilities for human transport of species in the region are likely to have occurred for a long period of time.

Roesel's bush cricket, *Metrioptera roeselii* (Hagenbach, 1822) (Orthoptera: Tettigoniidae), is an insect whose natural distribution extends across mid and northern Asia to Europe. In Europe, the cricket is mainly distributed in the central and eastern parts of the continent (Maas *et al.*, 2002). However, during the last 130 years, the natural range of *M. roeselii* has been extended by several long-distance colonization events and its populations have been recorded in Sweden, Denmark and England (Albrecht, 1963; Ahlén, 1995; Bavnhøj, 1996; Simmons & Thomas, 2004; Karjalainen, 2009). These populations are geographically isolated from the main distribution (Fig. 1) and are of unknown origin, although one of the biggest isolated populations in Sweden is thought to have been introduced through the transport of hay from Finland (de Jong & Kindvall, 1991). Interestingly, the first records of observation for several of these isolated sites (Fig. 1) do not coincide with the main pattern of expansion in continental Europe where the main range of *M. roeselii* has expanded westwards and northwards in recent years. Long-winged individuals (usually approximately 1% of the population) are capable of flying and are believed to disperse at most tens of kilometres (cf. Simmons & Thomas, 2004; Hochkirch & Damerau, 2009; Wissmann *et al.*, 2009). However, active flight across large water bodies appears unlikely and could only occur through passive aerial or water transport (Warne & Hartley, 1975; Gangwere & Llorente, 1992; Ashmole & Ashmole, 1997), or by human transport (Wagner, 2004; Whinam *et al.*, 2005; Kenis *et al.*, 2009). Thus, dispersal over large distances through active flight is not expected to explain the observed range expansion of this species.

The aim of our study was to increase the understanding of the colonization history of *M. roeselii* in northern Europe and to clarify the expansion pathways to sites that we consider to be far outside its natural range and dispersal potential. For these purposes we used existing biological information for the species, data on the establishment time in the specific regions, and genotypic data from analyses of mitochondrial DNA (mtDNA) cytochrome oxidase subunit I (*COI*) gene sequences and a set of polymorphic microsatellite markers. The acquired data and phylogeographical analyses, based on both the maternally-inherited mtDNA and nuclear markers, provide indications of the likeliest sources and colonization pathways of *M. roeselii* in northern Europe.

MATERIALS AND METHODS

The study species

Metrioptera roeselii is a bush-cricket that inhabits a wide range of grassland habitats, where it feeds on plant matter and small insects (Ingrisch & Köhler, 1998). *Metrioptera roeselii* has a univoltine or semivoltine life cycle. In northern Europe individuals reach maturity in summer (July) with a body length of 14–18 mm; adult males stridulate from July to October (almost continuously during the day if the weather is warm and sunny). The song is characteristic, making the adult males easy to detect. The females lay their eggs in hollow grass stems or other substrates and the nymphs hatch in spring, the first or second year after they are laid. The species has two wing forms: short-winged forms that disperse mainly through walking and long-winged forms that are capable of active flight (individual flight performance has not been measured, but macropterous individuals have been found up to 19 km from their source; Hochkirch & Damerau, 2009). It has been experimentally shown that macropterism (the development of long wings) can be environmentally induced (Poniatowski & Fartmann, 2009). The nymphs go through six instars before they are fully developed. Previous research has suggested that the expansion of *M. roeselii* may cause the displacement of a native orthopteran species (Berggren & Low, 2004), but its impact on the insect community as a whole is largely unknown.

Population sampling

Using hand-nets, we collected in total 768 *M. roeselii* individuals (513 males and 255 females) from 38 sites along the Baltic Sea coast, northern Europe (53°53′–65°49′ N, 08°34′–26°53′ E), between August and September 2008 (Fig. 1). We collected 16–24 individuals from 32 of the sites; however, at the other six sites sampling success was substantially lower and only 1–6 individuals were collected (hence data for

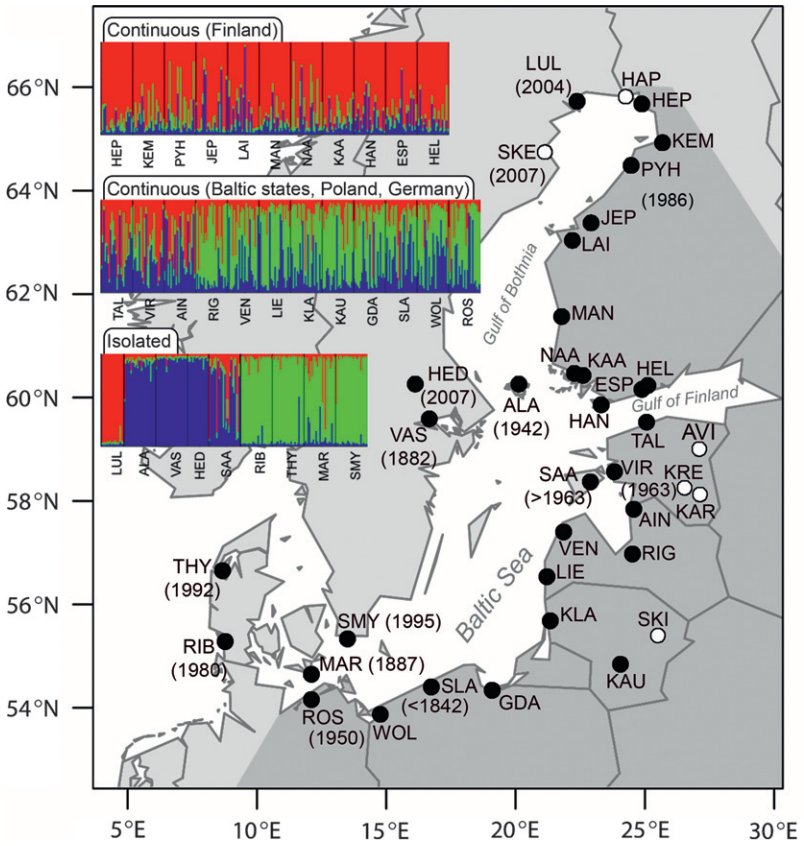

Figure 1 Sites (circles) where *Metrioptera roeselii* was sampled in northern Europe for phylogeographical analyses. Sites marked with white circles were only used in the mtDNA analyses (see Table 1 for site labels). The dark grey area on the map represents the area where *M. roeselii* is abundant and occurs as a continuous population, while isolated populations occur in the light grey areas. The bar plots show results from a cluster analysis of microsatellite data as implemented in STRUCTURE where each individual is represented by a single line broken into coloured segments in proportion to four inferred clusters. The year of the first published record or unpublished observation of the species in the area is shown for each isolated population and a subset of the sites within the continuous range.

these sites were only included in the mtDNA analyses). Ten of the sampled populations (in Sweden, Denmark, and the Baltic Sea islands of Åland and Saaremaa) were considered to be isolated, as they were separated from the species' continuous range by at least 100 km of land or 50 km of sea. The classification of the isolation thresholds are based on the known yearly dispersal distance of the species. The distance separating the isolated populations from other inhabited sites are much greater than the maximum reported annual dispersal distances of long-winged individuals (Hochkirch & Damerau, 2009; Wissmann *et al.*, 2009), and 100-fold longer than those of short-winged individuals (de Jong & Kindvall, 1991). For all isolated populations, except the population in central Sweden, samples were collected in a single area. However, in central Sweden sets of samples were collected from two sites (VAS and HED; Table 1), due to a rapid and recent expansion of *M. roeselii* from one locality close to the harbour of Västerås (VAS; where it was first observed in 1882), to its present day coverage of 15,000 km^2 (http://www.artportalen.se). The second sampling site in central Sweden (HED) is situated at the current edge of the species' range within this region. We also sampled sites at the edge of the continuous range of *M. roeselii*, at approximately 100–200 km intervals along the Baltic Sea coasts of Finland, Estonia, Latvia, Lithuania, Poland and Germany. To enable analysis of potential human-mediated aspects of dispersal we sampled sites close to major seaports in the region. Of our sampled individuals, 1–3 were macropterous at seven of the sites, while at the majority of sites (31) we did not find any long-winged individuals. This represents an average frequency of 1.7% long-winged individuals in total.

As *M. roeselii* is easily identified in the field due to its species-specific stridulation and unambiguous morphological characteristics (Ingrisch & Köhler, 1998), we gathered the earliest possible dates of the initial establishment of isolated populations (and hence their minimum ages) from published reports and databases (Albrecht, 1963; de Jong & Kindvall, 1991; Ahlén, 1995; Bavnhøj, 1996; Karjalainen, 2009; http://www.artportalen.se/; Fig. 1).

MtDNA sequence analyses

We obtained DNA from muscle tissue of the bush-cricket's hind femur using the DNeasy Blood & Tissue Kit (Qiagen, Inc., Valencia, CA) following the manufacturer's instructions, and we sequenced a maximum of four haphazardly chosen males and females (1–8 individuals) per site (Table 1). A fragment of the mitochondrial *COI* gene was amplified, using the general insect primer pair C1-J-2183 (5′-CAACATT TATTTTGATTTTTTGG-3′) and TL2-N-3014 (5′-TCCAATG CACTAATCTGCCATATTA-3′, Simon *et al.*, 1994), in 20 μL reaction mixtures containing: 0.2 μM of each primer, 300 μM of each dNTP, 2.5 mM $MgCl_2$, 1 × *Taq* DNA buffer (Qiagen, Inc.), 0.5 μg/μL BSA, 1 × Q-solution, 1 U of HotStar Taq DNA polymerase (Qiagen, Inc.) and 1 μL of genomic

Table 1 Geographical positions and distributions of mtDNA *COI* haplotypes in each (continuous and isolated) sampled northern European population of *Metrioptera roeselii*.

ID	Site (country code)	Lat N	Long E	*n**	Haplotypes # (*n*)†
Continuous populations					
HAP	Haparanda (SE)	65°49′	24°02′	2	07 (2)
HEP	Hepola (FI)	65°41′	24°39′	24	01 (1), 11 (3), 15 (4)
KEM	Kempele (FI)	64°56′	25°27′	24	02 (2), 15 (3)
PYH	Pyhäjoki (FI)	64°29′	24°15′	24	01 (2), 07 (3), 19 (1), 34 (1)
JEP	Jeppo (FI)	63°22′	22°42′	24	03 (1), 07 (3), 16 (1), 17 (2), 18 (1)
LAI	Laihia (FI)	63°02′	21°59′	24	01 (1), 02 (1), 07 (3), 27 (1), 28 (2)
MAN	Mäntyluoto (FI)	61°34′	21°34′	24	07 (4), 32 (4)
NAA	Naantali (FI)	60°28′	22°03′	24	07 (5), 14 (1), 33 (1)
KAA	Kaarina (FI)	60°26′	22°23′	24	07 (5), 14 (1), 19 (1), 20 (1)
HEL	Helsinki (FI)	60°14′	24°52′	24	07 (4), 11 (2), 14 (1)
ESP	Espoo (FI)	60°10′	24°38′	24	02 (1), 05 (2), 06 (1), 07 (3)
HAN	Hanko (FI)	59°52′	23°05′	24	03 (1), 07 (2), 09 (2), 10 (2), 11 (1)
TAL	Talin (EE)	59°31′	24°49′	24	01 (2), 04 (2), 52 (2), 53 (1), 54 (1)
AVI	Avinurme (EE)	59°00′	26°51′	2	01 (1), 04 (1)
VIR	Virtsu (EE)	58°34′	23°35′	24	03 (1), 44 (1), 57 (1), 58 (1), 59 (1), 60 (1), 61 (1), 62 (1)
KRE	Kärevere (EE)	58°16′	26°17′	1	03 (1)
KAR	Karilatsi (EE)	58°08′	26°53′	6	03 (1), 21 (3), 22 (1)
AIN	Ainaži (LV)	57°50′	24°20′	24	01 (7), 02 (1)
VEN	Ventspils (LV)	57°24′	21°39′	24	01 (1), 03 (4)
RIG	Rīga (LV)	56°59′	24°18′	24	03 (2), 08 (1), 38 (3), 39 (1)
LIE	Liepāja (LV)	56°32′	21°01′	24	03 (1), 29 (1), 30 (1), 31 (1)
KLA	Klaipėda (LT)	55°41′	21°08′	24	03 (1), 25 (1), 26 (2)
SKI	Skiemonys (LT)	55°24′	25°16′	2	03 (2)
KAU	Kaunas (LT)	54°51′	23°50′	24	03 (2), 08 (3), 23 (2), 24 (1)
SLA	Sławno (PL)	54°24′	16°34′	24	07 (1), 08 (2), 23 (2), 48 (1), 49 (1), 50 (1)
GDA	Gdańsk (PL)	54°20′	18°54′	24	03 (4), 08 (3)
ROS	Rostock (DE)	54°10′	11°58′	24	03 (1), 08 (1), 40 (2), 41 (1), 42 (2), 43 (1)
WOL	Wolin (PL)	53°53′	14°37′	24	03 (3), 08 (2), 63 (1), 64 (1), 65 (1)
Isolated populations					
LUL	Luleå (SE)	65°44′	22°10′	17	17 (8)
SKE	Skellefteå (SE)	64°45′	20°55′	2	47 (2)
ALA	Åland (FI)	60°16′	19°56′	24	03 (8)
HED	Hedemora (SE)‡	60°16′	15°57′	16	08 (5), 12 (1), 13 (1)
VAS	Västerås (SE)‡	59°35′	16°29′	24	08 (6)
SAA	Saaremaa (EE)	58°22′	22°41′	24	23 (1), 44 (3), 45 (2), 46 (1)
THY	Thyholm (DK)	56°39′	08°34′	24	35 (3), 55 (3), 56 (2)
SMY	Smygehuk (SE)	55°20′	13°22′	24	03 (7), 51 (1)
RIB	Ribe (DK)	55°17′	08°40′	24	35 (4), 36 (1), 37 (2)
MAR	Marielyst (DK)	54°39′	11°58′	24	08 (8)

*populations with 16–24 sampled individuals were genotyped at nuclear microsatellite markers, and a 676 bp fragment of the *COI* gene was sequenced in 1–8 individuals per population

†the GenBank accession numbers for 65 haplotypes are JX041527–JX041591

‡these two sites represent one isolated population in an area where the species has rapidly expanded.

Country abbreviations: DE, Germany; DK, Denmark; EE, Estonia; FI, Finland; LT, Lithuania; LV, Latvia; PL, Poland; SE, Sweden.

mtDNA (*c.* 100 ng μL^{-1}). The polymerase chain reaction (PCR) conditions consisted of initial denaturation (95 °C, 5 min) followed by 45 cycles of strand denaturation (94 °C, 30 s), primer annealing (47 °C, 30 s) and extension (72 °C, 90 s), then a final extension step at 72 °C for 10 min. The PCR products were purified using ExoSAP-IT (Affymetrix, Inc., Santa Clara, CA), then sequenced using a BigDye Terminator Sequencing Kit with both the forward and reverse primers, and analysed in an ABI 3730XL Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA). Sequences were controlled for accidental amplification of contaminating agents by alignment against sequences published in GenBank (http://www.ncbi.nlm.nih.gov/).

After removal of potentially low quality end sections of the sequenced fragment we aligned sequences by the Clustal W algorithm (Thompson *et al.*, 1994). In order to control for possible silent nuclear copies of the mtDNA *COI* gene (so-called NUMTs) (Lopez *et al.*, 1994), we translated the sequences into amino acids and checked for unexpected stop codons and controlled each sequence chromatogram for presence of double peaks (Song *et al.*, 2008). The identities and frequencies of haplotypes were determined using DnaSP

5.0 (Librado & Rozas, 2009). To assess the relationship between haplotypes, we constructed a minimum spanning haplotype network in TCS 1.2 (Clement *et al.*, 2000), and analysed the genetic relationships between populations in three ways. As the sample size differed between sites, the number of haplotypes was rarefied with a rarefaction calculator (available at http://www.biology.ualberta.ca/jbrzusto/rarefact.php) prior to the analyses of geographical structures so that the number of haplotypes was calculated from four sequences per site. To infer the number of genetic clusters we used a Bayesian clustering procedure as implemented in BAPS 5.1 (Corander *et al.*, 2008). We ran 10 replicates for each level of K, up to $K = 29$, and used only clusters that had at least 10 individuals present within them, using five reference individuals, and repeating the admixture analysis 20 times per individual. We then tested the strengths of the associations within and between the identified clusters in an analysis of molecular variance (AMOVA) using ARLEQUIN 3.5 software (Excoffier *et al.*, 2005). A Bayesian inference was implemented also for phylogenetic reconstruction in MRBAYES 3.2 (Ronquist *et al.*, 2012). We set an evolutionary model to the GTR substitution model with gamma-distributed rate variation across sites and a proportion of invariable sites. Markov chain Monte Carlo (MCMC) methods were used: four independent chains were run twice for one million replicates and sampled every 100 generations from which first 2500 generations were discarded as burn-in. The remaining trees were used to calculate a 50% majority-rule consensus topology. Branches with posterior probabilities > 95% were considered well supported.

To test for demographic expansion events in the sampled populations (Ramos-Onsins & Rozas, 2002) we ran two tests, Fu's F_S (Fu, 1997) and Fu and Li's F^* (Fu & Li, 1993) using simulations in ARLEQUIN 3.5 and DNASP 5.0, respectively. These tests determine if haplotypes are present in expected frequencies if the populations are in mutation-drift equilibrium (Wright–Fisher model) or if there are signs of processes that distort the pattern (such as population expansion or heterogeneity in mutation rates). A negative value of F^* is a sign of background selection while a negative estimate of Fu's F_S is evidence for an excess number of alleles compared with the expected based on the observed number of pairwise differences, as would be expected from a recent population expansion (however, selective sweeps can also give rise to the same signal).

Microsatellite analyses

For nuclear markers, DNA was isolated by the Chelex 100 (Bio-Rad Laboratories, Inc., Hercules, CA) extraction method (Walsh *et al.*, 1991), and used (at 20–40 ng μL^{-1} concentrations) to screen seven microsatellite loci: Metroe05, Metroe07, Metroe08, Metroe19, Metroe20, Metroe24 and Metroe27 (Kaňuch *et al.*, 2010). The protocol used for multiplex PCRs has been described by Kaňuch *et al.* (2010). The fluorescent labelled PCR products were separated by capillary electrophoresis in an ABI 3730XL Genetic Analyzer and we edited the electropherograms in PEAK SCANNER 1.0 (Applied Biosystems).

We tested for the presence of null alleles, effects of stuttering and large allele dropout using MICRO-CHECKER 2.2.3 (van Oosterhout *et al.*, 2004). Significant departure from Hardy–Weinberg (HW) equilibrium ($P < 0.05$ after false discovery rate corrections; Benjamini & Hochberg, 1995) was calculated from multiple tests using Q-VALUE software 1.1 (Storey, 2002). We calculated the allelic richness (AR) in FSTAT 2.9.3.2 (Goudet, 1995) and expected heterozygosity (H_E) and observed heterozygosity (H_O) with GENEPOP 4.0 (Rousset, 2008).

Pairwise F_{ST} estimates, corrected for null alleles by the ENA method using FREENA software (Chapuis & Estoup, 2007), were tested using a G-test, with strict Bonferroni correction in FSTAT 2.9.3.2. We also calculated D_{est} values, which account for alleles alternatively fixed in different populations (see Jost, 2008), whereas F_{ST} only considers levels of heterozygosity in different samples. D_{est} was calculated as a harmonic mean across all loci in SMOGD 1.2.5 (Crawford, 2010). We also tested for significant correlations between genetic and geographical distance for both estimators using a Mantel test and regressed pairwise estimates of $D_{est}/(1 - D_{est})$ or $F_{ST}/(1 - F_{ST})$ against ln distances between the populations (Rousset, 2008).

We applied two independent methods to analyse nuclear phylogeny and possible colonization routes of *M. roeselii* in northern Europe. First, clustering procedure implemented in STRUCTURE 2.3.3 (Pritchard *et al.*, 2000), was used to infer the number of distinct genetic clusters and to assign individuals to them. The simulation was run using an admixture model assuming K (unknown) populations that have different allele frequencies at a set of independent loci. The burn-in was set to 10,000 and was followed by 100,000 MCMC iterations. All runs were repeated five times for each number of possible clusters (K) ranging from 1 to 10. We identified the stable K solutions through novel correlation algorithms implemented in the package CORRSIEVE (Campana *et al.*, 2011) using an R statistical environment (R Development Core Team, 2008). Q-matrix correlations (average maximum correlation coefficient and the rows-and-columns method) yields information about overall stability of K solution's and helps to identify anomalous runs. Having established that any value of K is reproducible, we considered which value of K had the best data support. Second, isolated populations were assigned to possible founders in the continuous distribution of *M. roeselii* by GENECLASS 2.0 software (Piry *et al.*, 2004) where founder scores were calculated by the allele frequency-based method (Paetkau *et al.*, 1995).

RESULTS

Mitochondrial DNA diversity and genetic structure

We found high mitochondrial diversity in *M. roeselii* and no signs of NUMTs. Analyses of the 676 bp *COI* gene sequence

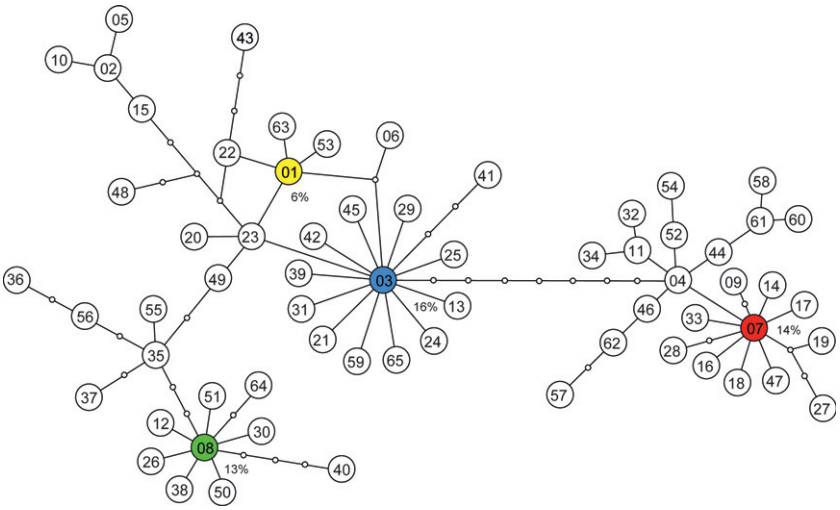

Figure 2 A minimum spanning haplotype network based on a 676 bp fragment of the mtDNA *COI* gene in *Metrioptera roeselii* sampled in northern Europe. Each node represents one mutational step. The four haplotypes that are shared by most individuals in the study are coded by colour (congruently with Fig. 3a).

revealed 73 (10.8%) variable sites with 65 unique haplotypes (GenBank accession numbers JX041527–JX041591) in 245 individuals from 38 populations. The four most common haplotypes were shared by almost half of the individuals (49% in total), and they made a major contribution to the structure of the three haplotype-groups in the minimum spanning haplotype network (see Fig. 2). The haplotype network showed a clear star-shaped pattern and Fu's F_S values were significant ($P < 0.02$) in several populations, not only in the isolated populations (see Appendix S1 in Supporting Information). On the other hand, Fu and Li's F^* values were significant in few sites, thus not indicating background selection to cause the observed pattern. Thus, the combined results indicate that the species has expanded recently across the whole investigated area.

Haplotype diversity varied with latitude and the distribution of haplotypes indicate that most of isolated populations were founded from the southern part of the investigated continuous area (Fig. 3a). Only the most recently founded populations in northern Sweden appear to originate from Finland as inferred from the Bayesian analysis (Fig. 3b). The Bayesian analysis found that the optimal number of clusters was $K = 3$, having a probability of 0.999, and the clusters obtained supported the geographical occurrence of the four most common haplotypes (Fig. 3a). The AMOVA confirmed the association of the identified clusters with the highest variation found among clusters (49.2%) and the lowest among sites within these clusters (12.0%, Table 2). Pairwise F_{ST} values were also significant ($P < 0.001$, Table 3) for all cluster combinations. A phylogenetic tree which plotted all sampled individuals identified two main genetic lineages. With the exception of the Baltic island population SAA, individuals of isolated populations clearly originated from either southern or northern genetic lineages of the continuous area (see Appendix S2).

Microsatellite diversity and genetic structure

Multilocus genotypes were obtained from 753 individuals with high scoring success (95.1%), indicating that the DNA material and methods were suitable for genetic analysis of the sampled populations. All seven microsatellite loci were highly polymorphic showing moderate level of heterozygosity and the number of alleles per locus ranged from 10 to 38 (Table S1 in Appendix S3). There was significant departure from HW equilibrium in most of the populations (6–86% per locus) due to heterozygote deficiency; this pattern was found across alleles of all sizes, indicating the presence of null alleles in all loci (1–15% per locus).

The microsatellite data verified the patterns observed in the mtDNA analysis although the pattern was not as clear. This is not unexpected as not all mutations in single sequence repeat (SSR) markers will appear as unique alleles due to homoplasy and substitutions in the flanking regions. Thus, our microsatellite estimates can be considered as more conservative than the true values. All but three pairwise F_{ST} estimates were significant (*G*-test, $P < 0.05$ after Bonferroni correction) and high levels of genetic differentiation were also found using the harmonic mean of the differentiation estimator (D_{est}) (Table S2 in Appendix S3). Pairwise D_{est} differences were on average 25% (range 2–70%; see Table S3), but there was a considerably higher level of differentiation within the group of isolated populations compared with the samples collected in the continuous area (Mann–Whitney *U*-test; $Z = 8.36$, $P < 0.001$). Using a Mantel test, we found a significant isolation-by-distance correlation in all three combinations of pairwise comparison (Fig. S1 in Appendix S3), i.e. within sample pairs in the continuous distribution area of species distribution ($r^2 = 0.39$, $P < 0.001$), between isolated and continuous sample pairs ($r^2 = 0.09$, $P < 0.001$) and within isolated sample pairs ($r^2 = 0.22$, $P = 0.012$). However, the variance was considerably larger for the isolated populations, and the correlation between the isolated sites disappeared when the most geographically and genetically (see later) distant site LUL (far north) was excluded ($r^2 = 0.08$, $P = 0.199$ after removal of LUL).

The *Q*-matrix correlations using both the maximum average correlation and the rows-and-columns criteria ($R = 0.99$, $P < 0.05$) revealed stable solutions for $K = 2$–5. As detailed

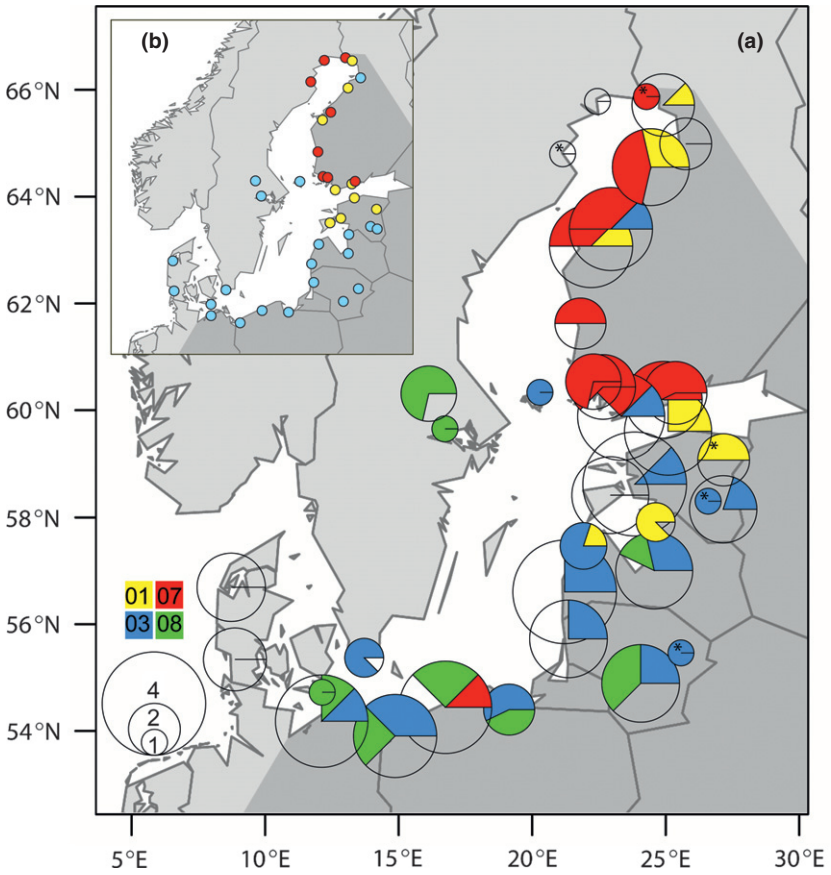

Figure 3 (a) Distribution of the four most common haplotypes in samples of *Metrioptera roeselii* in northern Europe based on mtDNA *COI* sequences. The haplotypes 01, 03, 07 and 08 are indicated by colour codes. The size of a circle represents the number of haplotypes in a population, rarefied to represent four sequences per site (*, the real numbers of haplotypes are shown for populations with less than four sequenced individuals). (b) The geographical distribution of the genetic clusters (yellow: cluster 1; red: cluster 2; blue: cluster 3) identified in the Bayesian clustering model implemented in BAPS (see Fig. 1 for site labels).

inspection of outputs of the rows-and-columns method found some anomalous runs in higher *K*-values (4–5), the optimal number of clusters inferred by STRUCTURE was set as $K = 3$ (Fig. 1). The sites from the continuous area of the *M. roeselii* distribution were divided into two main clusters separated by the Finnish Gulf (one north and one south of the Gulf). Only LUL clearly clustered with Finnish populations, as is indicated in the bar plots (Fig. 1). Although we did not obtain information in detail, other isolated populations were most probably founded from the southern continuous range. The Baltic island Saaremaa (SAA) appears to be founded from nearby Estonia. Much stronger genetic structure in the isolated populations than in the continuous range suggests restricted gene flow and/or a founding event with limited propagule size (Fig. 1). The allele frequency-based assignment of the isolated populations to their most likely origins supported previous inference and similarly indicated that the majority of founders originate from the southern continuous area of the *M. roeselii* distribution (Table 4). In the first rank assignment, isolated populations had, in all cases except one, very high likelihoods of the founder scores (> 95%). The distances between isolated populations and their inferred founders range between 57 and 589 km and in

Table 3 Average number of pairwise differences between genetic clusters (upper diagonal) and within cluster (diagonal elements) and F_{ST} values (lower diagonal) between clusters defined in BAPS based on mtDNA *COI* variation in *Metrioptera roeselii*. All F_{ST} values significantly differ from zero at $P < 0.001$, according to tests with 1000 permutations. Sites included in each cluster are shown in Fig. 3b.

Cluster	1	2	3
1	7.29	6.16	9.11
2	0.228	2.07	11.68
3	0.371	0.684	4.54

Table 2 Partitioning of mtDNA *COI* haplotype variation at different hierarchical levels in samples of *Metrioptera roeselii* from northern Europe, based on an analysis of molecular variance (AMOVA) including three clusters identified in a Bayesian analysis of population structure (BAPS). Sites included in each cluster are shown in Fig. 3b.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation indices
Among clusters	2	357.7	2.344 Va	49.2%	$\Phi_{CT} = 0.237$*
Among sites within clusters	35	192.2	0.572 Vb	12.0%	$\Phi_{SC} = 0.613$*
Among individuals within sites	207	381.8	1.845 Vc	38.8%	$\Phi_{ST} = 0.492$*

*$P < 0.001$, according to significance tests with 1023 permutations.

Table 4 The most likely founder sources of the isolated populations from the continuous area of *Metrioptera roeselii* distribution in northern Europe based on microsatellite data analysed in an allele frequency-based assignment test (GENECLASS) including the geographical distance between sampled sites (see Table 1 for site labels). Likelihood of the founder score (%) is shown in parentheses.

Isolated population	1st rank	2nd rank	3rd rank
SAA	VIR, 57 km (100.0)		
RIB	WOL, 414 km (99.9)	SLA, 514 km (0.1)	
THY	WOL, 492 km (100.0)		
MAR	WOL, 193 km (96.4)	SLA, 298 km (3.6)	
SMY	SLA, 230 km (100.0)		
ALA	TAL, 285 km (99.8)	HAN, 180 km (0.1)	LAI, 327 km (0.1)
VAS	SLA, 577 km (52.9)	TAL, 470 km (47.1)	
LUL	KAA, 589 km (100.0)		

several cases were not from the closest potential site (Fig. 1). For example, both the western populations in Denmark and the population in central Sweden appear to originate from northern Poland, and the most northern isolated site in Sweden has a most likely origin in southern Finland.

DISCUSSION

Analyses of both mtDNA haplotypes and microsatellite data suggest that at least some of the isolated populations are founded by individuals that originate from distant locations and not from the nearest possible ones within the continuous species range. For example, haplotypes found in the population VAS belong to a haplogroup that only includes sites located in the southern region of the continuous area and the assignment test of microsatellite data shows an equal likelihood of origin in both the southern and central continuous region (Table 4, Appendix S2).

The limited ability of *M. roeselii* to cross geographical barriers (Simmons & Thomas, 2004; Hochkirch & Damerau, 2009; Holzhauer *et al.*, 2009; Wissmann *et al.*, 2009), combined with the likely colonization pathways found in this study and the timing of specific events (see below), suggest that the isolated populations were established, at least to some extent, through passive and vector-borne introductions rather than active natural dispersal. In continental Europe, the range of *M. roeselii* has expanded westwards and northwards, and the rates of expansion have increased during the last 30 years (Maas *et al.*, 2002; Hochkirch & Damerau, 2009; Wissmann *et al.*, 2009). If the isolated populations we examined had been colonized only by natural processes in accordance with this general pattern of expansion, we would expect that they originated from the closest sites in the species' continuous range, at dates consistent with the species' natural expansion. This relationship, however, was not supported when the natural expansion chronology of *M. roeselii* was compared with the establishment time of the isolated populations. The population in central Sweden, for example, was founded approximately 100 years before the first individuals of *M. roeselii* colonized the same latitudes of the continental part of its range in Estonia and Finland. Similarly, the Åland population seems to have been established earlier than those at corresponding latitudes in Finland (Albrecht, 1963; Karjalainen, 2009), and the oldest isolated population in Denmark is more than 50 years older than the nearest possible source population (*c.* 50 km distance across the sea) in Germany (Maas *et al.*, 2002). On the other hand, *M. roeselii* was reported along the Baltic coast in Poland in the middle of the 19th century (von Siebold, 1842), and this date coincides with the time when it first appeared in Denmark and central Sweden. The genetic data, combined with the known expansion chronology of *M. roeselii* support the hypothesis that the colonization of the isolated sites resulted from human-mediated introductions, and that colonization of the Baltic Sea coast in the continuous species range was a prerequisite of this process (for dating of records see von Siebold, 1842; Albrecht, 1963; de Jong & Kindvall, 1991; Bavnhøj, 1996; Maas *et al.*, 2002; Karjalainen, 2009; Fig. 1).

We cannot, however, completely disregard natural long-distance dispersal through passive aerial transport by wind currents of long-winged individuals (e.g. Gangwere & Llorente, 1992; Ashmole & Ashmole, 1997; Gatehouse, 1997) or by eggs in floating material on water (Warne & Hartley, 1975). However, single individuals (a gravid female or a couple of eggs) experiencing such transport have a very low probability of establishing a viable population (Berggren, 2001). Also, if such dispersal events were effective we would expect a clearer connection between timing of expansion and establishment of isolated populations. Therefore, we argue that a more efficient vector of passive transport is more likely. Successful introduction of related orthopterans has been mediated by hay transport (Wagner, 2004). We suggest that transport of eggs laid in grass stems to new areas, in hay or other agricultural products, may explain the establishment of *M. roeselii* populations in the isolated areas examined in our study. Unintended transport of insect species via plant material is a well-known vector for pest transport and establishment in new regions (Kenis *et al.*, 2009), and cargo transport is known to facilitate alien species establishment in remote areas (Whinam *et al.*, 2005). The fact that all isolated populations are situated close to the coast or larger harbours, where active human transport networks exist, leads us to believe that establishment by human assistance is a likely vector of spread. His-

torical trading with livestock and cereals is documented and known to be common among the harbours in the Baltic Sea. For example, the region where *M. roeselii* first occurred in central Sweden and from which genetic data suggest it originated, has exchanged agricultural goods, horses and livestock since the 18th century (Bäckström, 1924; Montelius, 1993).

The overall congruence between the patterns inferred by nuclear and mitochondrial markers in our study is characteristic for cases when species expand into new areas (Brito, 2007). The genetic distances calculated between isolated populations and their inferred founders do not indicate ongoing gene flow, as the level of differentiation is higher within the group of isolated populations compared with all populations (cf. Haag *et al.*, 2006). The significant correlation we found between genetic and geographical distance for the isolated versus continuous sites appears to be consistent with natural processes (Epperson, 2003). However, this pattern is also expected for human-aided transports, as trading is more likely to occur among close than among more distant harbours.

Knowledge about colonization history provides an important understanding of factors affecting the establishment success, the relationship of these factors to specific species' traits, and the evolutionary capacities of a species. A recent study of *M. roeselii* found gradual phenotypic variation in isolated populations along a latitudinal gradient in northern Europe (Cassel-Lundhagen *et al.*, 2011), while no morphological gradient was apparent at corresponding latitudes among individuals within its continuous distribution area. Cassel-Lundhagen *et al.* (2011) suggest that the isolated position at the edge of the species' range prevented swamping gene flow from populations adapted to other conditions, and thus allowed offspring in the isolated populations to adapt more readily to local environmental conditions (García-Ramos & Kirkpatrick, 1997; Lenormand, 2002). This study enabled us to identify the origins of the isolated populations of *M. roeselii*, and this information will allow further insights to be gained into the influence of evolutionary history and contemporary gene flow on establishment success and adaptability (Schulte *et al.*, 2012). A characteristic of successful invading species is that isolated populations do not suffer from founder events or genetic bottlenecks (Dlugosch & Parker, 2008). The ecological knowledge of *M. roeselii*, in combination with its continuous range expansion, and the new insights into its colonization history, suggest that *M. roeselii* is a useful model species for future studies on the evolution of invasiveness (Berggren *et al.*, 2001; Simmons & Thomas, 2004; Hochkirch & Damerau, 2009).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Results of Fu's F_S and Fu and Li's F^* neutrality tests in *Metrioptera roeselii* populations including associated *P*-values.

Appendix S2 Bayesian phylogenetic tree of 245 individuals of *Metrioptera roeselii* sampled along the Baltic Sea coast based on mtDNA *COI* sequences inferred by MrBayes.

Appendix S3 Genetic diversity of *Metrioptera roeselii* populations (Table S1), pairwise genetic differences between populations (Table S2), and a plot illustrating the genetic isolation of populations by geographical distance (Fig. S1).

BIOSKETCHES

Peter Kaňuch is interested in various aspects of animal ecology and evolution. This study forms part of his postdoctoral research at the Swedish University of Agricultural Sciences.

Åsa Berggren is interested in factors that influence species dispersal and the effects of environmental factors on species invasion patterns.

Anna Cassel-Lundhagen is interested in how geographical barriers and ecological gradients affect dispersal patterns and evolutionary processes at both local and global scales.

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