

# Genetic patterns reveal an old introduction event and dispersal limitations despite rapid distribution expansion

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**Abstract** Identifying sources of range expansions after an introduction event and understanding the species dispersal are essential for effective management of invasive species. In a unique study system we investigated the spread and distribution of genetic diversity subsequent to a known colonization event and in the light of the well-known biology of the rapidly expanding Roesel's bush-cricket (*Metrioptera roeselii*), a species that is non-native in Sweden. Using eight microsatellite markers we analyzed genetic variation in 837 individuals collected at 29 sites across the species total range in central Sweden to verify the species local origin and to determine how the species known dispersal biology affect the population genetic structure throughout its range. We found that unique allelic richness was highest in a site approximately 16 km southwest of the previously suggested site of establishment, pointing towards a site of introduction close to a royal stud farm from where it is known that animals have been imported from Europe. Despite the species rapid expansion, genetic diversity in the core of the distribution was higher than in the populations at the range margin. Bayesian cluster analyses also

revealed that genetic structuring was more pronounced in the range margin, indicating the occurrence of dichotomous dispersal behaviour of the species with occasions of rare long distance events. Our study shows that good sampling design and appropriate choice of genetic markers can help to identify species local origin and explain genetic patterns that arise during range expansions.

**Keywords** Colonization · Founder effects · Population genetic structure · Orthoptera · Expansion pattern

## Introduction

Alteration of habitats and increased international trade are factors that contribute to the colonisation success of exotic species with potentially cascading effects on native species persistence, composition of local communities and ecosystem functioning (Sax et al. 2005; Suarez and Tsutsui 2008; Keller and Taylor 2010). Early intervention is crucial to successfully manage the expansion of non-native species in a region and this is aided by knowledge of the species origin, its ecology and its dispersal behaviour (Hulme 2006). Unless deliberate and post-introduction censuses are done, species colonisations and spread are initially difficult to detect because they usually consist of rare events and/or occur over large spatial and temporal

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scales (Puth and Post 2005). As a result, range expansions remain usually unnoticed until the species has become locally abundant (Sakai et al. 2001). Identifying the source of introduction, dispersal routes and the patterns of spread are essential for understanding the dynamics of species range expansions and the development of effective management strategies (Puth and Post 2005; Wilson et al. 2009; Ronnås et al. 2011).

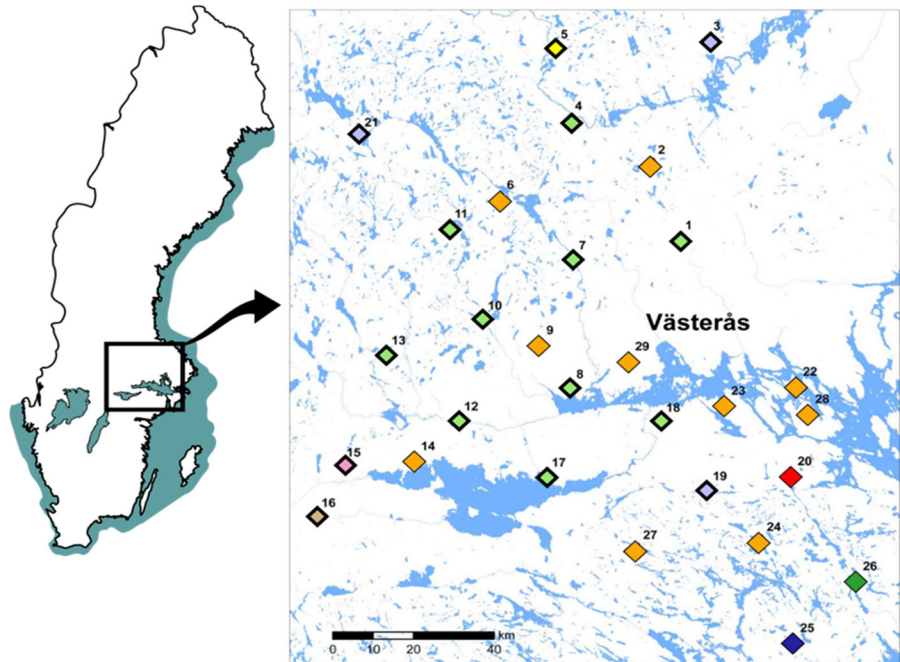
However, studying the distribution change of non-native insects over large spatial and temporal scales has its challenges due to the insects small body size, rapid reproduction and often complex dispersal behaviour. The use of neutral molecular markers and population genetic inferences has become a popular approach to investigate the history of populations and quantifying dispersal patterns (Brown 2004; Lawson Handley et al. 2011). Spatial and temporal variation of genetic structure arises as new populations are founded during range expansion events (Ibrahim et al. 1996) and are shaped by migration of individuals between populations. The amount of genetic variation in newly founded populations depends on the number and origin of founding individuals and subsequent immigrants (Le Corre and Kremer 1998; Dlugosch and Parker 2008; Kaňuch et al. 2014). Further, subsequent changes in genetic diversity within and among populations are influenced by the combined effects of the species dispersal behaviour and environmental factors. If a species spreads gradually and in a stepping-stone model fashion (Wright 1943; Kimura and Weiss 1964), one expects to find a correlation between the geographical distance separating populations and the genetic distance estimated from genetic markers. This is referred to as an isolation-by-distance pattern (Wright 1943). A more complex dispersal behaviour of a species is expected to result in a weak isolation-by-distance pattern and a spatially more complex genetic structure. One example of complex dispersal is the combination of long distance dispersal events by single colonizers that create genetic structures with populations having low and altered diversity due to local founder effects (Nichols and Hewitt 1994; Bialozyt et al. 2006) combined with short distance and frequent dispersal, as shown in dimorphic insects (Roff 1986).

There is a need to couple specific biological traits with historical events in order to fully understand population genetic patterns of expanding species

where the biological knowledge is less well known (Kirk et al. 2013). In order to add to the knowledge of how introductions to a new area and subsequent expansion we used a unique study system including a population of an introduced and rapidly expanding species (*Metrioptera roeselii*) where the history is well known (de Jong and Kindvall 1991; Preuss et al. 2011; Kaňuch et al. 2013) and where the species biology and dispersal behaviour has been extensively studied (Berggren et al. 2001; Berggren 2004; Simmons and Thomas 2004; Hochkirch and Damerau 2009; Holzhauser et al. 2009; Lange et al. 2010). This combination allows us to investigate fundamental population genetic processes and a non-native species dispersal patterns during range expansion (Kirk et al. 2013).

*Metrioptera roeselii* was first sighted in Sweden in 1882 near the city of Västerås at the shore of Lake Mälaren. The species was assumed of having been introduced via sea cargo (de Jong and Kindvall 1991) which is supported by findings that trace the species origin to northern Germany and/or Poland (Kaňuch et al. 2013). In Sweden *M. roeselii* went largely unnoticed for nearly 100 years and local spread was first noted in 1970 (Idar 1973). Comprehensive surveys were conducted during 1989–1990 and 2008–2010 resulting in detailed documentation of range expansion in the region (de Jong and Kindvall 1991; Preuss et al. 2011). Over the last 20 years the species has continued to spread rapidly at an estimated rate of 1–3 km/year (Preuss et al. 2014) resulting in an observed shift of the range margin by more than 50 km (Preuss et al. 2011). By 2010, the species distribution in the Mälaren region (59°35'N, 16°30'E) covered an area of approximately 120 × 140 km (Fig. 1). *Metrioptera roeselii* is a wing dimorphic species with dichotomous dispersal behaviour (Simmons and Thomas 2004; Poniatowski and Fartmann 2011). Most individuals (usually ca. 98 %) are short-winged and disperse short distances by walking and jumping (Vickery 1965; Wissmann et al. 2009). However, favourable weather conditions (mild springs and hot summers) and high population densities may trigger the development of long-winged individuals that can colonize distant habitat patches (Simmons and Thomas 2004; Gardiner 2009). The potential impact of introduced *M. roeselii* on the native insect communities or ecosystem functions is yet largely unknown although there are indications of competitive dominance (cf. Berggren and Low 2004). Despite the

**Fig. 1** Geographic location and genetic structure of 29 *Metriopectera roeselii* populations sampled in the Mälaren region (59°35'N, 16°30'E), Sweden. The *site number* corresponds to the population number (Pop) in Table 1. Västerås (Pop 29) was previous to this study the assumed site of introduction and Ullvi (Pop 8) is the inferred site of introduction. The *color of the squares* depicts the ten genetic clusters identified by *BAPS*. The *thickness of the squares outline* illustrates the two genetic clusters identified by *STRUCTURE*



limited evidence of negative impact on the native biota, this population constitute a useful system in which basic processes of human mediated colonisations and invasions can be studied (Allendorf and Lundquist 2003; Dlugosch and Parker 2008).

In this study we used neutral molecular genetic markers to identify *M. roeselii* first site of establishment and source of range expansion in central Sweden, and to investigate if the observed dispersal behaviour is mirrored in the genetic population structure as expected from theoretical modelling (Ibrahim et al. 1996; Bialozyt et al. 2006). We analysed the genetic variation and differentiation among 837 individuals from 29 locations sampled across the species total range in central Sweden (Fig. 1). We used eight polymorphic microsatellite markers to address the following three hypotheses: (1) *M. roeselii* was originally introduced to central Sweden at Västerås harbour and levels of genetic diversity are highest at this location. (2) Newly founded populations at the periphery of the range show lower levels of genetic diversity due to founder effects from long-distance dispersal events by long-winged individuals. (3) Subsequent and frequent short-distance dispersal by short-winged individuals reduces the genetic structure among populations situated in more central parts of the range.

## Methods

### Study species

*Metriopectera roeselii* (Hagenbach, 1822) (Orthoptera: Tettigoniidae) is a small (12–18 mm) bush-cricket commonly found in grasslands of central and northern Europe (Bellmann 2006). The species is expanding its range in several European countries (Netherlands: Kleukers et al. 1997; England: Thomas et al. 2001; Germany: Hochkirch and Damerau 2009; Wissmann et al. 2009). In northern Europe *M. roeselii* is both uni- and semivoltine, reproducing between July and September and overwintering as eggs in hollow grass stems and plant material (Marshall and Haes 1988). *Metriopectera roeselii* is an omnivorous generalist that prefers tall grassland habitats. In the agricultural landscape it colonizes extensively grazed pastures, leys, grassy field margins, ditches, and road verges (Marshall and Haes 1988; Berggren et al. 2001). Forests, crop fields and intensively grazed pastures are considered to be unsuitable habitat and urban areas are usually avoided by the species (de Jong and Kindvall 1991; Ingrisch, Köhler 1998; Wissmann et al. 2009). Short-winged individuals move on average 7.8–9 m (mean daily movement distance) and

**Table 1** Population (*Pop*) information including sample size (*n*), number unique alleles (*Ua*), mean allelic richness (*AR*), mean private allelic richness (*PAR*), unbiased gene diversity (*UHe*) and fixation index (*F<sub>IS</sub>*) for 29 populations of *Metrioptera roeselii* collected across the species whole range in central Sweden

Pop	Location	Latitude	Longitude	n	Ua	AR	PAR	UHe	F <sub>IS</sub>	<i>p</i>
1	Husta	59.85723	16.63562	30	1	6.76	0.08	0.638	0.028	0.227
2	Hede	60.04005	16.52009	27	0	6.75	0	0.656	0.028	0.235
3	Österfärnebo	60.3371	16.7747	30	0	5.93	0	0.619	-0.051	0.930
4	Avesta	60.14769	16.15113	30	1	7.05	0.08	0.666	0.076	0.019
5	Vikbyn	60.3207	16.08895	30	0	4.80	0	0.556	-0.011	0.636
6	Åvestbo	59.95737	15.84697	27	0	6.70	0	0.641	0.056	0.066
7	Färmansbo	59.81064	16.16533	30	0	7.00	0.13	0.629	0.054	0.073
8	Ullvi	59.49999	16.15129	30	2	7.37	0.26	0.684	0.067	0.024
9	Tangsta	59.60645	16.02073	27	0	6.47	0.01	0.637	0.037	0.175
10	Vallbricka	59.66619	15.76266	30	0	6.14	0.05	0.626	0.034	0.177
11	Sotebo	59.88748	15.6146	30	1	7.15	0.08	0.681	0.046	0.080
12	Fellingsbro	59.42199	15.76256	30	2	6.73	0.21	0.616	0.036	0.143
13	Fageräng	59.58087	15.3401	30	0	6.44	0	0.594	0.004	0.491
14	Lillkyrka	59.32613	15.47224	28	0	6.49	0.05	0.664	0.096	0.013
15	Örebro NW	59.30931	15.14814	30	0	5.63	0.04	0.585	0.010	0.415
16	Örebro SW	59.18721	15.04156	30	0	4.10	0	0.476	0.022	0.298
17	Vi	59.28672	16.04147	30	1	6.94	0.17	0.630	0.035	0.187
18	Torshälla	59.41697	16.54147	30	0	6.86	0.04	0.620	0.033	0.197
19	Eklången	59.25055	16.74073	30	0	7.41	0	0.662	0.064	0.036
20	Åkerstyrebruk	59.28369	17.11083	28	0	4.90	0	0.536	-0.033	0.804
21	Rävsnäs	60.1067	15.2122	30	0	5.69	0	0.579	0.018	0.302
22	Fröta	59.49978	17.14231	27	1	6.07	0.13	0.656	0.071	0.035
23	Ekeby	59.45619	16.827	27	0	6.83	0.01	0.633	0.055	0.062
24	Ullsta	59.12613	16.97154	28	1	7.03	0.19	0.64	0.016	0.319
25	Torsberga	58.88071	17.10718	28	0	4.83	0	0.587	0.136	0.002
26	Mölnbo	59.0268	17.38291	28	0	4.09	0.08	0.497	-0.089	0.975
27	Grabhed	59.11063	16.43566	27	1	6.35	0.17	0.652	0.033	0.199
28	Nursa	59.4327	17.19358	27	0	6.41	0.05	0.605	0.021	0.290
29	Västerås	59.5661	16.409	28	0	6.55	0	0.668	0.039	0.157

*p* values of within-sample Hardy–Weinberg test ( $\alpha = 0.005$  after Bonferroni correction) is also included. Västerås (Pop 29) was according to previous studies the assumed site of introduction and Ullvi (Pop 8) is the inferred site of introduction

maximum 172 m/day (Kindvall et al. 1998). The measured mean activity radius of long-winged individuals is 144.4 m (Poniatowski and Fartmann 2011). High population density and favorable weather conditions can trigger the development of long-winged morphs that are capable of flight dispersal of up to 19 km and an average displacement of individuals of 10.8 km (Hochkirch and Damerou 2009).

### Sampling scheme

During July and August 2010, we collected 27–30 short-winged individuals of *M. roeselii* per site from 29 locations across the species distribution around Lake Mälaren. The locations were situated from the core to the margin of the species distribution. The average minimum straight line (Euclidean) distance between sampling sites was 20 km and the most

marginal populations were situated around 85 km from the assumed introduction site near the city of Västerås (Table 1; Fig. 1).

### DNA extraction and genotyping

We isolated DNA from the femur muscle of 837 individuals using the *Chelex 100* (Bio-Rad Laboratories, Inc.) extraction method (Walsh et al. 1991). We used 13 microsatellite markers that have been specifically developed for *M. roeselii*: Metroe05, 07, 09, 08, 19, 20, 24, 27 (Kaňuch et al. 2010) and MR2-16, 2-42, 3-12, 3-24, 3-34 (Holzhauer and Wolff 2005). The microsatellite primer pairs were arranged in two multiplex polymerase chain reaction (PCR) set-ups (Supplementary Table 1). Loci were amplified in 10 µl PCR reactions containing 1 µl DNA (~20 ng/µl), 0.3 µM of primer mixture (forward and reverse) of each locus and 2×KAPA2G Fast HotStart Ready-Mix (Kapabiosystems) under the following conditions: 95 °C for 1 min, 35 cycles at 95 °C for 10 s, 56 °C for 10 s, 72 °C for 10 s and final extension at 72 °C for 1 min. Forward primers were 5'-end labelled with different fluorescent dyes (Supplementary Table 1), enabling unambiguous detection of similar sized PCR-products from co-amplified loci when analyzed on an ABI3730XL automated capillary sequencer using Gensize LIZ500 (Applied Biosystems) as a size marker. The alleles were scored using GENEMAPPER version 4.1 (Applied Biosystems). Loci with unusual peak patterns and individuals with rare alleles were analyzed twice to minimise scoring errors.

### Data analyses

All loci were checked for the presence of stuttering, large allele drop out and null alleles using MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2006). We tested for linkage disequilibrium (LD) and deviation from Hardy–Weinberg equilibrium (HWE) at each locus (exact probability test with 1 000 iterations) and across all loci (Markov chain method) using GENEPOP 4.0.1 (Raymond and Rousset 1995), adjusting the significant threshold values for multiple tests using a sequential Bonferroni correction (Rice 1989). We excluded five loci (Metroe05, 07, 09, 20, MR3-12) from the subsequent analyses due to high frequency of null alleles and unclear banding patterns. Further

analyses were based on eight loci (Metroe08, 16, 19, 24, 27, MR2-42, 3-24 and 3-34). For those of the remaining loci that showed evidence of null alleles in some populations, we calculated adjusted allele frequencies using the Brookfield1 correction method (Brookfield 1996) implemented in MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2006). Genetic diversity per population was described by calculating unbiased gene diversity ( $U_{He}$ ) and inbreeding coefficient ( $F_{IS}$ ) corrected for sample size in FSTAT 2.9.3 (Goudet 2001). Mean allelic richness (AR) and mean private allelic richness (PAR) standardized for sample size ( $N = 26$ ) were calculated using ADZE (Szpiech et al. 2008). Differences in levels of genetic variation between populations found within the core range versus the marginal were tested with a Mann–Whitney  $U$  test applied on the values averaged across loci. Populations found within a radius of 70 km were defined as core populations and those found further away (70–100 km) as range margin populations. To test for increasing variability in measures of genetic diversity and inbreeding from the centre of the range towards the margin, we used a linear regression with the residuals of the respective genetic parameters as explanatory variables.

We analyzed population differentiation by calculating overall and pairwise estimates of  $F_{ST}$  (Weir and Cockerham 1984) among sampling locations, conducting allelic permutation tests for each locus independently (10,000 permutations) in GENEPOP 4.01 (Raymond and Rousset 1995). Additionally to the traditional fixation index ( $F_{ST}$ ) we calculated the diversity index  $D_{est}$  (Jost 2008) using the web based program SMOGD v1.2.5 (Crawford 2010). Similar to  $F_{ST}$ ,  $D_{est}$  is a relative measure of differentiation which ranges from zero (no differentiation) to one (complete differentiation) but contrary to the former,  $D_{est}$  is a more accurate measure when overall divergence is large as it accounts for differences in allele identities. Simulations have shown that  $D_{est}$  outperforms  $F_{ST}$  over a range of sample sizes and for markers with different numbers of alleles, e.g. for highly variable microsatellite loci (Gerlach et al. 2010).

We conducted Mantel tests (Mantel 1967) in GENEPOP 4.01 (1000 permutations) (Raymond and Rousset 1995) to examine the relationship between the genetic distance measures  $F_{ST}$ ,  $D_{est}$  and the geographic distance between the sampling locations measured as the straight line (Euclidean) distance.



We used two different model-based Bayesian clustering methods, STRUCTURE v.2.2.3 (Pritchard et al. 2000; Falush et al. 2007) and BAPS v5.1 (Corander et al. 2003; Corander and Marttinen 2006) to identify the number of genetically distinct groups in the study region and to assign the sampled individuals to these groups. We first used STRUCTURE v.2.2.3 to test for the most likely number of genetic clusters (K) ranging from 1 to 15, assuming correlated allele frequencies and using sampling localities as prior information (LocPrior). We ran the simulations with a burn-in of 50,000 followed by 100,000 MCMC (Markov Chain Monte Carlo) iterations and with ten runs per K. To identify the most probable number of clusters we used the posterior probability of the data for a given K,  $\text{LnP}(D)$ , and  $\Delta K$  values as described by Evanno et al. (2005). Following the recommendations of François and Durand (2010) we accounted for admixture and ran spatially explicit models in BAPS v5.1. Because BAPS incorporates geographic information of the samples, we reanalyzed the data in BAPS v5.1 which uses a stochastic optimization algorithm (Corander et al. 2008a) rather than MCMC simulation as in standard Bayesian hierarchical modelling techniques, and automatically estimate the most probable number of genetic clusters (K). The analysis was done in two steps; in the first step we ran a spatial clustering of individuals to allocate each sample into its most likely genetic group using the geographic coordinates of the samples to create a Voronoi tessellation representing spatial population structure (Corander et al. 2008b). We tested values of K ranging from 1 to 15 genetic clusters and ran five iterations for each value of K. The most likely number of clusters was assessed by using changes in the logarithm of the maximum likelihood for each value of K and the Bayesian posterior probability (PP) computed for the ten best partitions (Corander et al. 2006). The PP value closest to 1 identifies the most likely number of genetic clusters supported by the data. In the second step, we used the results obtained from the spatial clustering of individuals to run an admixture model to estimate the proportion of an individual's ancestry contributed by each of the identified genetic groups (Corander and Marttinen 2006). We used a minimum group size of five individuals to construct admixture sets. Admixture coefficients for individuals were calculated after 50 iterations and following recommendations by Corander and Marttinen (2006), and

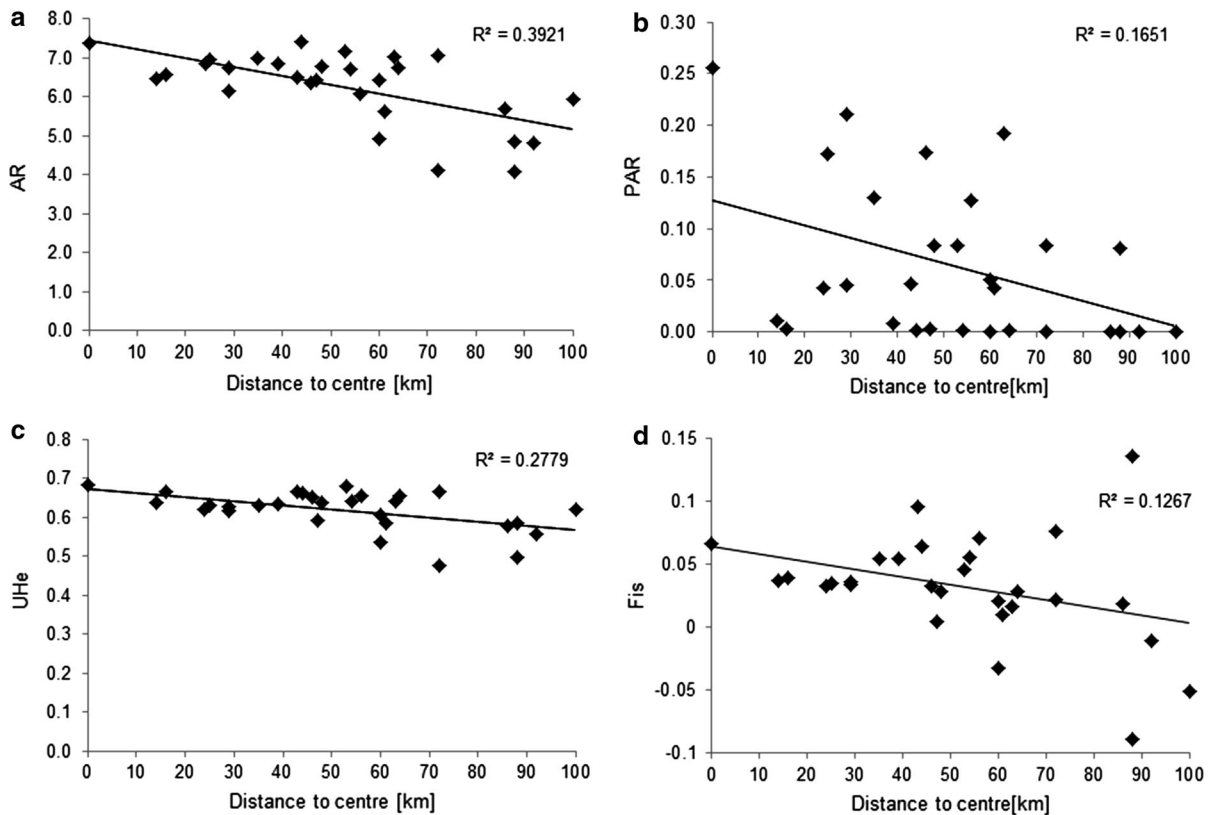
we used 200 reference individuals to calibrate our estimates.

We tested for signals of recent bottlenecks in the sampled populations, using the statistical approach of Cornuet and Luikart (1996) implemented in the program BOTTLENECK (Piry et al. 1999). The program generates the expected heterozygosity under mutation-drift equilibrium from the number of alleles at a locus and the sample size using different mutation models, and compares it to the average values of observed heterozygosity across all loci and populations. Following the recommendations of Piry et al. (1999), we chose the two phase mutation (TPM) model which applies to most microsatellite loci, with a variance of 30 and a proportion of stepwise mutation model (SMM) in TPM of 70 %. We ran 1000 iterations and due to the small number of loci (<20) we used the Wilcoxon sign-rank test to examine whether the observed heterozygosity deviated from the expected (Piry et al. 1999).

## Results

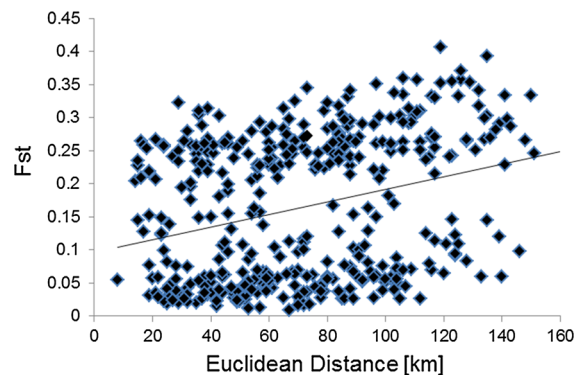
Three loci (Metroe19, MR2-42, and MR3-24) exhibited heterozygote deficiency and deviations from Hardy–Weinberg Equilibrium ( $p < 0.002$ ; GENEPOP global test) in several populations. This was likely due to the presence of null alleles since deviations were present across all allele sizes. We did not find evidence of linkage disequilibrium among any pairs of loci across all populations ( $p > 0.05$ ). Of the 29 populations analyzed, only one (Pop 25) showed a significant deviation from HWE ( $p < 0.006$ ) and an excess of homozygotes ( $F_{IS}$ ,  $p < 0.002$ ; Table 1).

Mean allelic richness (AR) per population was  $6.92 \pm 0.92$ , ranging from 4.09 to 7.41. Average gene diversity ( $U_{He}$ ) across all loci and populations was  $0.62 \pm 0.05$  and varied from 0.48 to 0.68 (Table 1). Populations sampled at the outer margin of the distributional area showed a lower AR, PAR and lower gene diversity ( $U_{He}$ ) than the average across all populations, but we found no signs of increased inbreeding (Mann–Whitney  $U$  test AR:  $p = 0.006$ , PAR:  $p = 0.023$ ,  $U_{He}$ :  $p = 0.013$  and  $F_{IS}$ :  $p = 0.321$  respectively) (Table 1; Fig. 2). Overall,  $F_{IS}$  values were low across all sampled populations (mean  $F_{IS} = 0.032$ ) ranging from  $-0.089$  to  $0.136$  (Table 1). Analysis of the residuals of the genetic measures



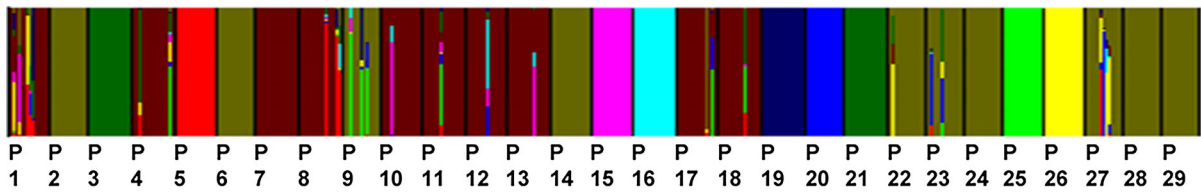
**Fig. 2** Genetic diversity in populations of *M. roeselii* sampled at an increasing distance from centre of distribution (Ullvi; Pop 8): **a** Allelic richness (AR), **b** Private Allelic richness (PAR), **c** Unbiased heterozygosity (UHe), and **d** Inbreeding coefficient ( $F_{IS}$ )

showed a positive correlation between distance from the range centre and variability around the mean for all estimates (AR:  $p = 0.012$ ,  $UHe$ :  $p = 0.003$  and  $F_{IS}$ :  $p = 0.009$ ) except for PAR, for which the correlation was negative (PAR:  $p < 0.001$ ) (Supplementary Fig. 1). Unique alleles (Ua) were mostly found in populations located in the centre of the distribution range. No unique alleles were found in populations at the marginal sites (Table 1; Fig. 1). Although genetic diversity in marginal populations was lower compared to the populations in the centre of the range, no evidence of recent population bottlenecks was found. Analyses of genetic variation among subpopulations using  $F_{ST}$  and  $D_{est}$  indicated the presence of moderate population differentiation (mean  $F_{ST} = 0.152$ ,  $SE = 0.052$ ; mean  $D_{est} = 0.173$  at  $p < 0.001$ ). Because  $F_{ST}$  and  $D_{est}$  were showing the same pattern of population differentiation we present only the results of  $F_{ST}$  (Fig. 3), which is the most commonly used measure in population genetic analyses. The



**Fig. 3** Correlation between geographic distance and genetic distance ( $F_{ST}$ ) of *M. roeselii* sampling sites in south central Sweden ( $R^2 = 0.124$ ,  $p < 0.001$ )

correlation of the geographic (Euclidean) distance between sites and pair-wise  $F_{ST}$  values of all 29 populations revealed a significant isolation-by-distance pattern ( $R^2 = 0.124$ ,  $p < 0.0001$ ) (Fig. 3).



**Fig. 4** Estimated number of genetic clusters and proportion of individual's ancestry in a given genetic cluster as inferred by BAPS ( $K = 10$ ) illustrated in different colored bars. Bar height represents probability of each individual belonging to a specific genetic cluster

Interestingly, the data points appear to cluster in two groups.

Bayesian cluster analysis using STRUCTURE indicated the presence of two genetic clusters while BAPS identified ten genetic clusters. Both analyses, STRUCTURE and BAPS, converge on the population structure found in the centre of the range, assigning populations to the same genetic cluster. Additionally, BAPS identified genetic divergence in the marginal populations (Fig. 1). Admixture analyses indicated limited amounts of gene flow among populations (Fig. 4).

## Discussion

Contra our first hypothesis, the number of unique alleles and level of allelic richness were not highest in the population at Västerås (Pop 29), which had been the assumed introduction site for *M. roeselii* in central Sweden based on historical observation records. No unique alleles were found in the Västerås population and genetic diversity was comparable to most parts across the sampled range (Table 1). Instead we found the largest number of unique alleles and highest levels of allelic richness (mean and private allelic richness) at Ullvi (Pop 8) situated 16 km southwest of Västerås. This suggests that *M. roeselii* rather started expanding from Ullvi and was detected in Västerås at an early stage of the colonization process. The same number of unique alleles but a lower level of allelic richness was found also in a site west of Ullvi (Fellingsbro, Pop 12) which also indicate an early establishment. Interestingly, the sampling site at Ullvi is situated only five km southwest of the royal stud farm of Strömsholm that was established already in the seventeenth century. Strömsholm was a central military riding school and also became a home to the National Swedish Horse Academy. Historical trade records of extensive horse

and cattle imports (Bäckström 1924; Montelius 1993) combined with recent genetic analyses (Kaňuch et al. 2013) suggest that *M. roeselii* was accidentally introduced to this region with hay that arrived with imports from northern Germany or Poland. Species which occupy human modified grassland habitats have an increased likelihood of being introduced to new locations via the transportation of agricultural crops (Wagner 2004). Since *M. roeselii* overwinters by laying its eggs in hollow grass stems and plant material (Marshall and Haes 1988), we suspect that individuals can be transported with plant materials over large distances. Human mediated dispersal is playing an important role in population expansion and genetic differentiation of other invasive species such as the spread of alien red swamp crayfish in China (Yue et al. 2010), Eurasian dreissenid mussels in USA (Brown and Stepien 2010), and the common wall lizard in Germany (Schulte et al. 2013).

Recurring animal imports from across the Baltic to Västerås and transport to farms in the vicinity appear to have resulted in multiple introductions of individuals as well as the introduction of different lineages of *M. roeselii* (Kaňuch et al. 2013). This is one possible explanation for the distinct genetic clusters we detected in our analyses. Species dispersal behaviour is also known to affect the genetic structure of populations and the spatiotemporal distribution of genetic variability (Holway and Suarez 1999; Wilson et al. 2009). *Metrioptera roeslii* is an example of a species with a stratified dispersal pattern, where early colonization and range expansion is driven by rare long-distance dispersal followed by frequent short distance dispersal ("diffusion") which shapes the population genetic structure (Shigesada et al. 1995; Ibrahim et al. 1996). This is manifested in our data by the two most widespread clusters (shown in yellow and green in Fig. 1) that are not found in well defined regions but rather, to some extent, intermixed. The



unique clusters at the range margins also support a pattern that agrees with rare long distance colonisation events.

The overall genetic pattern of the populations sampled around Lake Mälaren appears to reflect the species distribution history (de Jong and Kindvall 1991) and known dispersal biology (Ingrisch and Köhler 1998) very well. Similar pattern of population structure have been observed in other expanding populations in the species native range (Hochkirch and Damerau 2009; Wissmann et al. 2009) and in the UK (Simmons and Thomas 2004). The likelihood that *M. roeselii* performs long-distance dispersal appears to be low until environmental conditions are favourable and high population densities are reached (Simmons and Thomas 2004; Hochkirch and Damerau 2009; Poniatowski and Fartmann 2009). High propagule pressure and growing population densities in the centre of the species range near Lake Mälaren are likely to have triggered the development of long-winged individuals which were able to colonize distant habitat patches and contribute to the advance of the range margin.

The combination of recurring occasional long distance dispersal by long-winged individuals, frequent short distance movements by short-winged individuals and presumably human aided dispersal at any distance can also explain the genetic pattern of gradually declining genetic variation and increasing variability around the mean towards the range margin. Similar spatiotemporal patterns of genetic diversity have also been observed in other invasive species that rapidly expand their range (Dong et al. 2013, Schulte et al. 2013, Signorile et al. 2014).

The fact that we found low levels of genetic variation in the populations at the range margin suggests that they have been recently founded by a very small number of individuals. A recent study conducted by Kaňuch et al. (2014) confirms our findings that both allelic richness and unbiased expected heterozygosity in isolated populations are correlated with population age. The lack of evidence of recent bottlenecks in any of the sampled populations and only slightly elevated levels of homozygosity indicate that immediate consequences of founder effects are not traceable (Luikart and Cornuet 1998). It is likely that the populations at the range margin have not reached a demographic equilibrium yet and the genetic composition is expected to change with time as a consequence of genetic drift and gene flow (Bohonak

1999). *Metrioptera roeselii* ability to regain genetic diversity appears to contribute to the species successful range expansion in northern Europe (Kaňuch et al. 2014; Wissmann et al. 2009).

The moderately decreasing levels of genetic diversity with increasing distance from the core area and evidence of a weak but significant isolation-by-distance pattern in combination with the marginal pockets of low and altered variation are further indications of moderate levels of short distance gene flow distorted by rare long distance dispersal. The weak isolation-by-distance pattern may also suggest a presence of unidentified geographic barriers present in the landscape that restrict the movement of dispersing individuals. The effect of landscape configuration, presence of geographic barriers such as large bodies of water or other types of unsuitable habitat and frequent land use changes causing habitat fragmentation have been shown to affect species genetic structure in other regions (Diekötter et al. 2010, Holzhauser et al. 2006, Lange et al. 2010; Pierce et al. 2014). The landscape around Lake Mälaren consists of a mosaic of agricultural land, forests intersected by small streams, roads and settlements that create a mosaic of more or less permeable habitat and non-habitat. An intensified or altered land use may result in increased fragmentation of landscape that affects species distribution and dispersal.

The majority of *M. roeselii* individuals in the region around Lake Mälaren are short winged and have a limited movement radius. Despite the occasional occurrence of long-winged individuals and human mediated dispersal, the level of gene flow appears not be high enough to even out variation among populations of *M. roeselii* as we found a significant differentiation amongst populations. The combination of the species stratified dispersal (species dispersal behaviour and transport by humans) and landscape composition and configuration could be the explanation for the complex spatial genetic structure of *M. roeselii* around Lake Mälaren. The creation of new grassland habitats within the region can increase dispersal success, and this may result in an increase of genetic diversity and a reduction of genetic differentiation (Gibbs 2001; Lange et al. 2010) as was found in the more central areas around lake Mälaren.

In order to understand in detail what is shaping the genetic population structure and driving the species range expansion further analyses of the exact links

between landscape composition, dispersal behaviour and spatiotemporal aspects of genetic variation are needed. Technical and methodological advances will facilitate future genomic data analyses combined with analyses of complex landscape datasets, which will further improve our understanding of the continuous interplay between evolutionary adaptations, genetic drift and homogenizing gene flow during range expansions (Alleaume-Benharira et al. 2006; Kawecki 2008; Rice and Papadopoulos 2009; Bridle et al. 2010).

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