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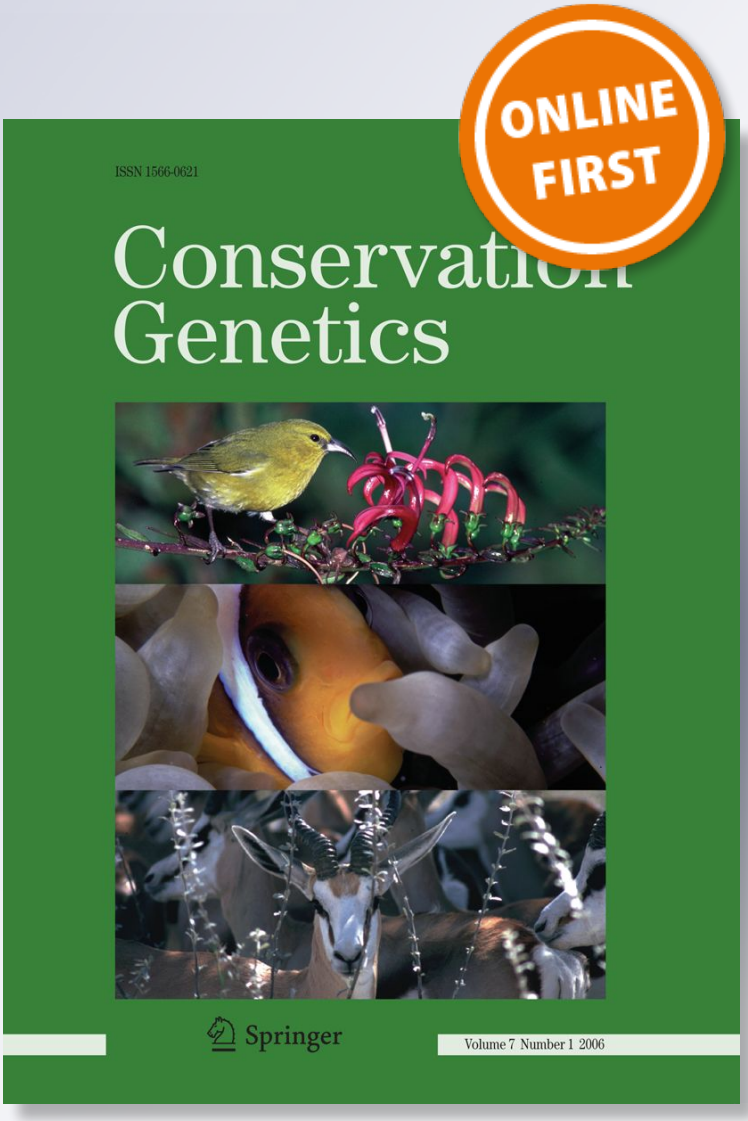

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RESEARCH ARTICLE

Genetic variation and population decline of an endangered hoverfly *Blera fallax* (Diptera: Syrphidae)

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Abstract Genetic diversity is one of several factors affecting extinction risk in vulnerable populations. In addition to informing conservation management strategies, data on genetic variability can also shed light on the recency and magnitude of historic bottlenecks. The pine hoverfly *Blera fallax* is one of the rarest invertebrates in the UK, known from just two sites in Scotland. It belongs to an often overlooked, species-rich community that is fundamental to forest function, the saproxylics (that depend on dead wood). To assist current conservation management for *B. fallax*, including captive breeding and translocations, it is important to know whether genetic factors will limit the success of recovery. Using 12 microsatellite loci, we compared the genetic variation in Scottish and Swedish specimens (Swedish populations are thought to represent a more outbred *B. fallax* population). As expected, the Scottish population showed significantly lower levels of polymorphism, expected heterozygosity and allelic richness than the Swedish population. Furthermore, significant genetic differentiation was found between the two *B. fallax* populations ($F_{ST} = 0.134$). We then used an allele frequency-based approach and a Bayesian coalescent-based method to assess genealogical history and detect recent changes in population size. Unexpectedly, data from not only the Scottish but also the Swedish population indicated a strong and relatively recent decline that was more pronounced in Scotland. We discuss the implications of our findings for future conservation management planning, the first undertaking of its kind for saproxylic species in Britain.

Keywords Syrphid · Pine hoverfly · Microsatellite · Population bottleneck · Population structure · Conservation

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Introduction

The conservation management of endangered species involves ensuring the survival of viable populations and increasing their abundance and distribution (Primack 1998). This requires knowledge of the behaviour and ecology of a species in order to identify the causes of decline and manage accordingly, but it also involves assessing genetic diversity in the frequently small, isolated and threatened populations, which can limit the adaptive potential of the species (Lande 1988). Populations with limited genetic diversity are more susceptible to environmental change and thus at greater risk of extinction (Frankham 1995, 1998, 2005). Where captive breeding and translocation play a role in management protocols, inbreeding effects and effective population size become particularly relevant issues (Leberg 2005). Reduced fitness caused by inbreeding has been demonstrated in numerous controlled experiments (Armbruster et al. 2000; Woodworth et al. 2002; Whitehorn et al. 2010) and in studies on

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wild populations (Brown and Brown 1998; Keller 1998; Saccheri et al. 1998). While the effects on different taxa and individual populations appear to vary (Elgar and Clode 2001), especially with respect to demographic and environmental stochasticity, inbreeding depression is considered pervasive enough to have a generally detrimental effect on population persistence (Keller and Waller 2002). Where populations are considered highly vulnerable, conservation management often involves introducing individuals from a genetically and demographically healthy population to improve fitness (Dowling et al. 2005; Edmands 2007; Biebach and Keller 2010). While this has shown to be highly effective across a number of studies (reviewed in Tallmon et al. 2004), such intentional hybridization can also lead to a subsequent reduction in fitness known as outbreeding depression (Templeton 1986; Lynch 1991; Edmands 2007), an effect that often becomes apparent in later generations (e.g., Armbruster et al. 2000; Aspi 2000). While evidence for outbreeding depression is scarce, it is important to consider this risk and assess genetic and adaptive similarity between populations intended for translocation and hybridization (Edmands 2007).

Our main objective in the current study was to investigate the genetic diversity of the UK endangered hoverfly *Blera fallax* (Linnaeus 1758) (Diptera, Syrphidae) by comparing a Scottish population with one in Continental Europe that, based on the distribution and condition of its pine wood habitat (Willis et al. 1998), is assumed to be less isolated and more panmictic (Rotheray, personal communication). Our data will facilitate population monitoring and the design of conservation strategies for *B. fallax* in Britain as well as help to assess the feasibility of translocation and captive breeding of *B. fallax* from elsewhere in Europe if a genetic 'rescue' attempt is necessary.

Historically, *B. fallax* was probably an early coloniser of the Caledonian pine wood habitat; the larva filter-feeds on microbes in rot-holes occurring in decaying roots and holes in the surface of stumps of Scots Pine, *Pinus sylvestris* L. (Rotheray and Stuke 1998; Rotheray and MacGowan 2000). This microhabitat develops due to heart-rot fungi softening heartwood that is often exposed when a tree falls or is felled. Based on survey results from five consecutive years, *B. fallax* over-winters at the larval stage and primarily has a univoltine life cycle (Rotheray et al., unpublished data). As a saproxylic species, *B. fallax* is an important bio-indicator of habitat quality and is part of a very diverse, species-rich group of organisms that play a vital functional role in forest ecosystems, and include a high proportion of threatened species (Speight 1989; Grove 2002; Jonsson et al. 2005; Lassauce et al. 2011). *Blera fallax* is found across the Palearctic as far as Japan and south as far as the Pyrenees and, based only on scant, intermittent records collected over the past 250 years, it is considered locally rare or declining wherever it has been recorded (Speight 2008). No detailed information on the distribution or health of these Palearctic populations exists. In Scotland *B. fallax* shares its habitat with at least 30 endangered taxa from several groups including Diptera (Rotheray et al. 2001), parasitic Hymenoptera, Coleoptera, fungi and lichens (Alexander 1988; Butler et al. 2002). *Blera fallax* is listed in the UK red data book as category 1 (endangered). It is a biodiversity action plan priority species and is one of 32 species listed in the species action framework, a Scottish natural heritage initiative that focuses on improving the status of species deemed significant to overall Scottish biodiversity (Scottish Natural Heritage 2007).

Based on historical pine pollen records and indications derived from fossils of colonizing arthropods during the Holocene epoch (Birks 1970; Bennett 1984; Whitehouse 2006), *B. fallax* has probably been isolated in Scotland since the last glaciation 7,000–10,000 years ago. Its geographic range underwent a severe decline from eight to two sites between 1950 and 2000 due to loss of habitat and changes in forestry management (Rotheray and MacGowan 2000). Larval counts and extensive habitat surveys indicate that just a few hundred individuals remain across both sites (MacGowan, personal communication). Furthermore, survey work during the past 5 years has failed to locate signs of *B. fallax* at one of these sites. The remaining population may be highly isolated, inbred and have limited dispersal ability. Therefore current conservation practices involve captive breeding and translocation to historically inhabited sites in Scotland where new habitat has been created. To assist this effort, we urgently need data on the effective population size and genetic diversity of the remaining population. In this context we sought to estimate the genetic diversity of Scottish *B. fallax*, to compare it with Swedish samples, and to assess the signs of recent demographic changes in both populations.

Methods

Sampling and DNA extraction

In October 2008, after extensive searches identified just one remaining locality for this species, 50 *B. fallax* larvae were collected from 40 pine rot-holes at Curr Wood in Strathspey, Scotland, UK (57°18′N, 3°39′W). No more than two larvae were collected from one rot-hole. These were reared to eclosion and bred in captivity as part of a captive breeding program (Rotheray 2010). Seventeen individuals that had endured substantial wing damage while in captivity were frozen upon death, while the rest were released at the collection site in Curr Wood in an attempt to minimize the impact of our sampling on the source population. Between

Fig. 1 Map showing locations of the Scottish (*left circle*) and Swedish (*right circle*) *B. fallax* populations used in the study

June and November 2009, 22 larvae and one adult *B. fallax* were collected by Hans Bartsch from a pine woodland site in Järfälla, Sweden (59°24′N, 17°52′E) (Fig. 1). We selected this site because of its similar latitude and its proximity to a colleague who could collect and identify specimens. No other extant sites were readily available for sampling at this stage. Although no detailed surveys of the current health of this population (or any other Palearctic population) exists, we expected based on the availability of habitat that the Swedish population should be larger. All Swedish larvae were frozen before being transferred to 90 % ethanol, and the adult female was pinned dry.

Whole larvae were used to extract genomic DNA. The hind legs were removed from adults and stored frozen as a reference collection in case of contamination or loss of samples, while the rest of the body was used for DNA extraction (Rotheray et al. 2011). Twelve species-specific microsatellite markers (HF_8RB, HF_S56, HF_WMK, HF_JRW, HF_C4A, HF_OH2, HF_0IY, HF_AN4, HF_5VB, HF_AMQ, HF_FCT, HF_RKX) developed from Scottish *B. fallax* were used for population genetic analysis (Rotheray et al. 2011). Polymerase chain reaction and genotyping was carried out as in Rotheray et al. (2011).

Statistical analysis

The statistical analyses to determine allelic richness, heterozygosity and population differentiation were performed with Fstat 2.9.3 (Goudet 1995), GenALEx 6.1 (Peakall and Smouse 2006) and Arlequin 3.11 (Excoffier et al. 2005). Population structure was inferred using a Bayesian clustering method implemented in STRUCTURE 2.3.3 (Pritchard et al. 2000), which allocates individual genotypes into groups (K number of populations) by an estimated membership coefficient Q for each individual based on allele frequencies at unlinked loci. An admixture model was used with default parameter settings. One to five possible populations (K) were tested; each run had a burn in period of 50,000 steps, followed by 100,000 steps of Markov chain Monte Carlo sampling, and was iterated five times for both populations together and for each separately. Convergence was checked by comparing the output between runs.

In order to detect population decline and find evidence for historical bottlenecks we used two methods. The first analysed allele frequencies in BOTTLENECK 1.2 (Piry et al. 1999). Here we used two models of mutation: the more conservative stepwise mutation model (SMM) and the less conservative infinite allele model (IAM) (Luikart and Cornuet 1998; Maudet et al. 2002). The second employed likelihood-based Bayesian methods that inferred population demographic history by coalescence from the full allelic distribution, implemented in MSVAR version 1.3 (Beaumont 1999). This assumes an ancestral effective population size N_1 that gradually changed to a recent effective population size N_0 at time T_a generations. We assumed a single generation per year (based on results from field surveys and larval growth studies, Rotheray et al., unpublished data), an exponential population size change and wide, log-normal distribution prior for the mean value of the demographic parameters across loci: mean 4 and variance 3 for N_1 and N_0; mean 3 and variance 2 for time since population size change T_a. The prior of the mutation parameter μ was set to a relatively low level of mean -4 and variance 0.5, based on the mixture of di, tri and tetranucleotidic repeat motif in the microsatellites used (Rotheray et al. 2011). A wide prior with a low mean is necessary due to the lack of empirical and independent data on *B. fallax* microsatellite mutation rate. All demographic and mutational parameters were allowed to vary among loci using the hierarchical model implemented in MSVAR (Beaumont 1999) and setting a mean of 0 and a variance of 0.5 for the variance parameters. Each Markov chain was run for 5×10^9 steps recording the parameter values every 50,000 steps for a total of 100,000 output lines. We ran five independent chains using different starting values to assess convergence using the Gelman-Rubin diagnostic (Gelman and Rubin 1992) implemented in the R package *coda* (Plummer et al. 2006), after cutting off the first 10 % of each chain as a burn in period. The chains were then combined to estimate the mode of the posterior density of the model parameters and their 90 % high probability density (HPD) using the R package *boa* (Smith 2007).

Results

Genetic variation

The percentage of polymorphic loci was 91.67 % in the Swedish population and 66.67 % in the Scottish

Table 1 Population statistics comparing Scottish and Swedish *B. fallax* populations per locus

			Scotland					Sweden				
Locus	F_{ST}	P F_{ST}	NA/private	AR	H_E	F_{IS}	P F_{IS}	NA/private	AR	H_E	F_{IS}	P F_{IS}
HF_8RB	−0.001	0.375	4/0	2	0.747	−0.271	0.972	4/0	4.00	0.758	−0.269	0.989
HF_C4A	0.148	0.010	2/0	3	0.337	0.130	0.537	2/0	2.00	0.511	−0.109	0.823
HF_JRW	−0.021	0.538	3/1	3	0.508	0.076	0.440	2/0	2.43	0.449	−0.066	0.786
HF_S56	0.104	0.000	3/0	2	0.683	−0.304	0.941	3/0	3.98	0.590	0.268	0.068
HF_WMK	0.154	0.005	2/0	3	0.059	0.000	–	3/0	2.98	0.503	0.139	0.333
HF_0IY	0.151	0.000	3/0	1	0.570	−0.032	0.556	6/3	5.34	0.763	−0.143	0.742
HF_5VB	0.078	0.063	1/0	2	0.000	*	*	3/2	2.37	0.237	−0.105	1.000
HF_AN4	0.155	0.000	2/0	1	0.487	−0.344	0.979	4/2	3.43	0.675	−0.229	0.950
HF_OH2	0.338	0.000	1/0	1	0.000	*	*	6/5	4.75	0.706	0.078	0.271
HF_AMQ	*	*	1/0	2	0.000	*	*	1/0	1.00	0.000	*	*
HF_FCT	0.034	0.248	1/0	2	0.000	*	*	2/0	1.82	0.125	−0.048	1.000
HF_RKX	−0.023	1.000	2/0	2	0.337	0.130	0.537	2/0	2.00	0.348	−0.257	1.000
Over all loci	0.134	0.000	2	2	0.298	−0.0884	0.537	3	3.16	0.491	−0.037	1.000

F_{ST} differentiation coefficient, *P* F_{ST} *p* value of observed F_{ST} under the assumption of panmixia, *NA* number of alleles and private alleles, *AR* allelic richness, H_E expected heterozygosity, F_{IS} inbreeding coefficient, *P* F_{IS} *p* value of observed F_{IS} under the assumption of panmixia

*Monomorphic—no *p* value due to calculated F_{IS} value of zero

population. The inbreeding coefficient (F_{IS}) across all loci in both populations showed no significant deviation from zero (Table 1), indicating a lack of evidence for non-random mating and further population substructure. After Bonferroni corrections, locus HF_8RB showed significant deviation from Hardy–Weinberg in the Swedish population and was excluded from further analyses.

The maximum number of alleles per locus varied from four (mean 2.08 ± 0.29 SE) to six (mean 3.12 ± 0.46 SE) in the Scottish and Swedish populations respectively. One private allele was found in the Scottish population while twelve were found in the Swedish (Table 1). Overall, expected heterozygosity (H_E) and allelic richness was significantly lower in the Scottish population (H_E 0.30 ± 0.08 SE; allelic richness 2.0 ± 0.26 SE) than in the Swedish population (H_E 0.49 ± 0.06 SE; allelic richness 3.3 ± 0.5 SE) (one-tailed Mann–Whitney; H_E and allelic richness $P = 0.05$) (Table 1).

Population genetic structure

Genetic differentiation between the two populations was significant ($F_{ST} = 0.134$, $P < 0.001$, Table 1). The STRUCTURE analysis revealed that the data were most likely to come from two populations (K = 2, results not shown) and clear segregation was found from the assignment test; 93 % of Scottish individuals were assigned to one population and 83 % of the Swedish individuals to the other (Fig. 2). No subdivision was found within either population; all individuals were assigned to each cluster (K = 2 to 5) with equal probability. This demonstrates an absence of immigration from other populations i.e. a lack of population substructure.

Inferences of population demographic history

Under IAM, both the Scottish and Swedish populations showed significant signs of recent bottlenecks (Wilcoxon test, one-tailed for heterozygote excess, Scottish $p = 0.039$ and Swedish $p = 0.009$) while under the more conservative SMM there was a marginally non-significant trend in the Scottish population (Wilcoxon test, one-tailed for heterozygote excess, $P = 0.055$), and no evidence for the Swedish population (Wilcoxon test, one-tailed for heterozygote excess, $P = 0.313$).

Using Bayesian methods (Beaumont 1999), we found a clear, strong signal for a population decline in both populations (Table 2). The five independent analyses of the two populations yielded convergent chains according to the Gelman–Rubin diagnostic (results not shown). The genetic data contained useful information to estimate the demographic parameters as shown by the posterior distributions that differ substantially from the prior distributions (Fig. 3a, b, c). As expected, there was no information concerning the mutation rate, as the posterior and the prior distributions for the mutation rate parameter are similar (Fig. 3d). This implies that the demographic parameter estimates depend on the mutation rate prior, which was confirmed by repeated analysis using different mutation rate priors (data not shown). Contemporary effective population size, N_0, is relatively smaller in Scotland (12 [0-266] individuals, mode and 90 % HPD) than in Sweden (80

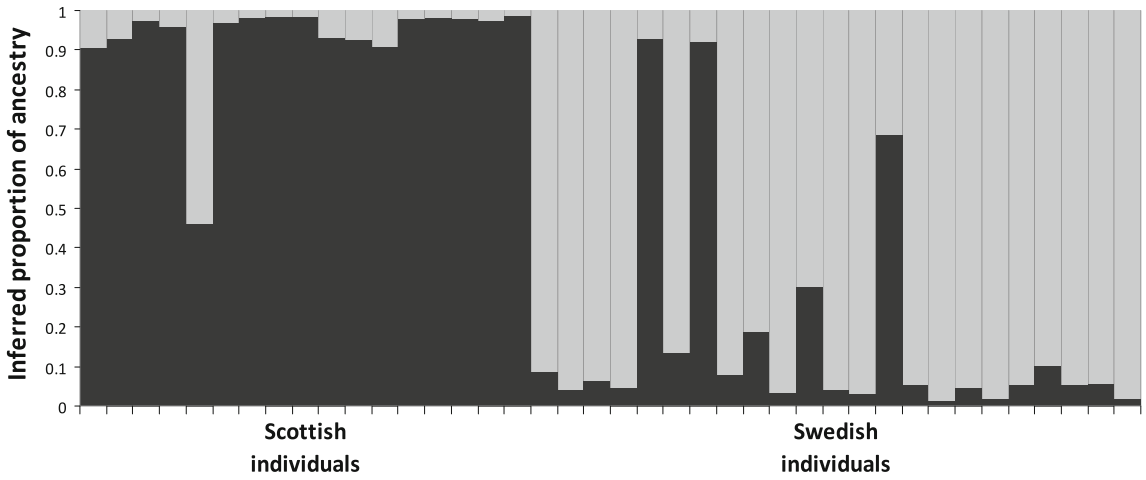

Fig. 2 *Bar* plot showing inferred population ancestry (membership coefficient Q) for two assumed clusters (K = 2) indicated by *dark and light grey bars* (*first 17 bars* are individuals sampled from the Scottish population, and the remaining 23 are Swedish)

Table 2 Posterior distribution estimates: the mode and the 90 % HPD interval in brackets estimated from the posterior density kernel of the log scale parameter (as showed in Fig. 1) back transformed in natural scale

Population	Contemporary effective population size (N_0)	Ancestral effective population size (N_1)	Time since population size change (T_a)	Mean mutation rate (μ)
Scotland	12 (0–266)	47,567 (5,456–4,46,937)	167 (3—3,159)	0.73 (0.12–4.46)
Sweden	80 (1–1,654)	31,169 (4,224–2,27,798)	344 (4–6,578)	0.76 (0.13–4.81)

N_0 and N_1 are expressed in number of individuals, T_a in years assuming a generation time of 1 year and μ in 10^{-4} mutations per generation per haploid genome

[1-1654]) (Table 2). These low estimates are more than two orders of magnitude smaller than the large ancestral effective population sizes for the two populations: 47,567 [5,456–446,937] and 31,169 [4,224–227,798] individuals for the Scottish and Swedish populations, respectively (Table 2). The time since population size change is slightly more recent for the Scottish population, 167 years ago [3–3,159], compared to 344 [4–6,578] for the Swedish population. The high magnitude of population size change, computed as $\log_{10}(N_0/N_1)$, of −3.49 [−5.46 to −2.27] and −2.39 [−4.62 to −1.43] for the Scottish and Swedish populations respectively, means that it is very unlikely that such high estimates result from a confounding effect such as population substructure or sampling bias alone (Chikhi et al. 2010). This likelihood is verified by the lack of substructure found by the STRUCTURE analysis within each population. These results clearly indicate a very strong and relatively recent decline in effective population size in both populations, which is more pronounced and probably more recent for the Scottish one.

Discussion

As expected, the geographically isolated population of *B. fallax* in Scotland has less genetic diversity than the population in Sweden. Moreover, the two populations are clearly genetically distinct. More unexpected was the finding that both the Scottish and Swedish population appear to have gone through a fairly recent and severe decline, which has direct consequences for the conservation of the species and suggests that *B. fallax* may be especially vulnerable to habitat fragmentation.

Genetic evidence for population isolation in Scotland is apparent through the allele frequency-based bottleneck analysis. The less conservative IAM model suggests that both Scottish and Swedish populations have gone through a recent bottleneck, whereas under the SMM model (which is considered more suitable for microsatellites; Luikart and Cornuet 1998), the test for the Scottish population was marginally non-significant (Wilcoxon Test: PSMM = 0.055), and there was no evidence for a bottleneck in the Swedish population. A conservative interpretation of these results would suggest the Scottish population has gone through a recent bottleneck (Luikart and Cornuet 1998). This initially confirmed our presumption that the population from Sweden would show less evidence for a decline due to greater habitat continuity. Swedish populations are less isolated and likely to experience immigration from surrounding localities, i.e. experience greater gene flow, and the habitat is considered to be less fragmented which facilitates gene flow. However, the Bayesian modeling

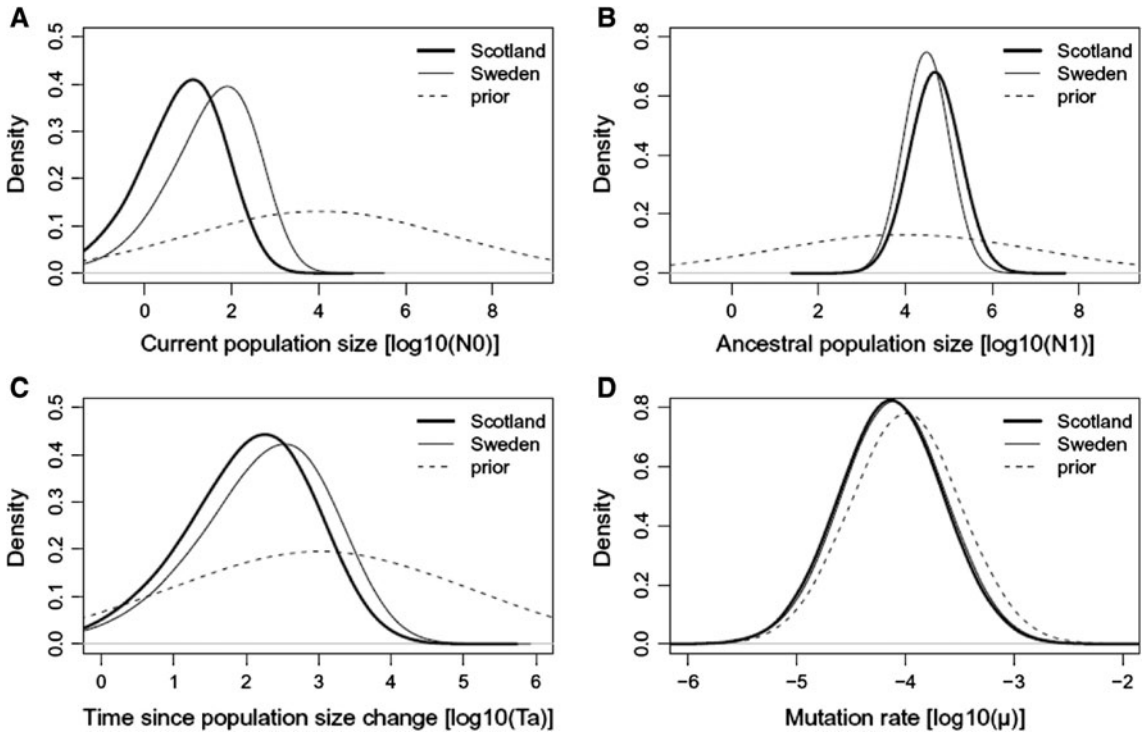

Fig. 3 Posterior density distributions for **a** current and **b** ancestral effective population size, **c** time since the population decline and **d** microsatellite mutation rate for Scottish and Swedish populations of *B. fallax*. The *dashed lines* represent the prior distributions of the parameters, the *thick*, *dark* and *thin*, *light grey lines* represent the Scottish and Swedish populations respectfully

method suggested that both populations have undergone a severe decline, which occurred approximately 200 years earlier in the Swedish population. This estimate accords reasonably well with human changes in land use in the 1700s; forest fires were repressed and woodlands were felled for construction and timber (Zackrisson 1977). It may have been forest fires, which happened on an ~80 year cycle (Zackrisson 1977) that caused an initial bottleneck in the population, or proceeding deforestation since fires were repressed. We would need more data about the past and current population and habitat structure in Sweden, and to sample more Swedish *B. fallax* populations in order to comment further. The discrepancy between the bottleneck analysis and the Bayesian inference is probably due to inefficient use of the genetic information for the former approach (Felsenstein 1992). Similar situations where the SMM failed to detect a bottleneck have been found in other studies (e.g., Olivieri et al. 2008; Craul et al. 2009). If the Bayesian analysis is correct, it indicates that *B. fallax* may be particularly vulnerable to habitat fragmentation and, as suggested by observations of the Scottish population, have limited dispersal ability even in pine woodlands that appear to be fairly well connected (Willis et al. 1998).

The Scottish population shows a more severe decline, estimated to have occurred more recently, approximately 167 years ago. This estimate is supported by the history of woodland management in Strathspey recorded for this period. Individuals sampled in Scotland are from a population that may have been restricted to a 200-ha pine plantation, Curr Wood, since the 1800s. This woodland was planted with native *P. sylvestris* in 1796 and was established woodland by 1858 (Dunlop 1993). Between 1750 and 1850, and during World War 1 and 2, substantial clear felling was carried out in Strathspey (Worrell and Dunlop 2003). While this may have provided a lot of *B. fallax* habitat at the time, i.e., numerous pine stumps left to decay, after a period of natural regeneration these areas were extensively re-planted and re-seeded which involved ploughing and up-rooting stumps (Dunlop 1994). Accidental fires associated with the felling process were frequent, and destroyed vast areas of woodland; a six day long fire in 1948 destroyed the woodland near to and surrounding Curr Wood, which has never recovered (Dunlop 1994). During this time Curr Wood survived and, while thinning continued periodically, it was left to regenerate naturally (Worrell and Dunlop 2003) which is probably how *B. fallax* became isolated but persisted there.

The more recent population decline and isolation in Scotland may explain the reduced genetic diversity when compared to Sweden, however occurring on the edge of a species' range can also have consequences for the genetic

diversity of populations (Hewitt 1996, 2000; Ibrahim et al. 1996). Such genetically impoverished populations have been found in many studies across taxa from European pool frogs *Rana lessonae* (Zeisset and Beebee 2001), and European hedgehogs *Erinaceus europaeus* and *E. concolor* (Seddon et al. 2001) to the grasshopper *Chorthippus parallelus* (Cooper et al. 1995) and the butterfly *Polyommatus coridon* (Krauss et al. 2004). The Scottish *B. fallax* population is on the edge of its West European range while the Swedish population is in the centre of its North Westerly range. Investigation into the genetic diversity of *B. fallax* across its European range will be necessary in order to explore this further.

Conservation and future research

The species' geographic range and the recent bottleneck may explain the reduced genetic diversity observed in the Scottish population, but we do not yet know the fitness consequences of this reduction. While evidence has shown reduced genetic diversity has a deleterious effect on fitness (Reed and Frankham 2003), the effect of inbreeding on fitness in wild populations is not only difficult to measure, it also varies a great deal depending on different evolutionary and environmental factors, such as past founding events, genetic purging or complex ecological associations (Hedrick and Kalinowski 2000). Ongoing monitoring of the Scottish population will be necessary in order to detect any detrimental effects. Since 2007, in an effort to recover *B. fallax* in Scotland, active conservation management has included captive breeding from the remaining Scottish population and relocation to historic native pine woodlands where rot-holes have been artificially created. While preliminary results for the translocation look promising (Rotheray 2010), the long and short-term effects this will have on genetic diversity, especially with regard to the number of individuals bred and subsequently translocated, are unclear. Due to lack of records and experts outside of Britain, sampling was only possible from one other population in Europe to compare with what appears to be the sole remaining UK population. Future work should assess and compare the diversity of populations across the Palearctic in order to better evaluate the condition of the Scottish population, as well as gain a better sense of *B. fallax* population genetic structure, effective population size and evolutionary potential. Sampling historic genetic data using museum specimens has been done successfully for butterflies and bumblebees (Harper et al. 2006; Strange et al. 2009; Lye et al. 2011), and we consider this to be another potential source of informative genetic data. Results from such studies may also clarify the probable viability of future relocations on newly founded Scottish sites. Maudet et al. (2002) showed through simulations that only a small number of immigrants from a well-differentiated, variable population are required to improve and sustain heterozygosity. However, if such translocations are to be carried out as part of ongoing conservation efforts, care should be taken with regard to possible adaptive differentiation across populations. The clear genetic distinction between the Scottish and Swedish populations is not unexpected, as they may have been separated for up to 10,000 years. It is possible that due to this separation and resulting divergence, the two populations may have become locally adapted, in which case hybridization of these stocks could have negative genetic repercussions (Templeton 1986; Lynch 1991; Edmands 2007). Translocation efforts using individuals from Sweden could fail due to them being maladapted, or could cause outbreeding depression. Evidence of outbreeding depression has been demonstrated for hybridizing populations of mosquito fish *Gambusia holbrook* just 100 m apart (Templeton 1986) and bark beetles *Xylosandrus germanus* 6 km apart (Peer and Taborsky 2005), and second generation fitness problems arose after crossing *Drosophila* from geographically isolated populations (Aspi 2000). Even though the evidence for such local adaptation causing outbreeding depression is relatively scarce compared to that for inbreeding, translocation of individuals for hybridization with those in Scotland should only be carried out if the population is clearly suffering from inbreeding depression.

Saproxylic organisms form a complex and specialized community of decomposers, fundamental to forest function (Speight 1989; Grove 2002; Schmuki et al. 2006). Modern forestry practices continue to overlook the importance of retaining dead wood, often opting for an over-managed, 'tidy' woodland system (Butler et al. 2002; Humphrey 2005; Humphrey et al. 2005; Lonsdale et al. 2008). Due to the limited and temporally unpredictable availability of dead wood and the dependency of many species on specific stages of its decay, many saproxylic populations are characteristically small and isolated, but often exhibit limited dispersal abilities (Speight 1989; Butler et al. 2002; Grove 2002; Ranius 2006). This causes particular susceptibility to adverse effects including woodland fragmentation (Jonsson 2003; Schmuki et al. 2006) and, especially where they depend more on plantations rather than natural mixed-age woodland, demographic fluctuations such as boom and bust cycles (Rotheray et al. 2009). For *B. fallax*, the microsatellite markers used here to assess genetic diversity can be further utilized by investigating fine-scale population genetic structure to better understand mating systems and dispersal ability, thus inform efficient conservation management strategies.

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