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Unconstrained gene flow between populations of a widespread epiphytic lichen *Usnea subfloridana* (Parmeliaceae, Ascomycota) in Estonia

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ABSTRACT

Few studies have investigated the genetic diversity of populations of common and widespread lichenized fungi using microsatellite markers, especially the relationships between different measures of genetic diversity and environmental heterogeneity. The main aim of our study was to investigate the population genetics of a widespread and mainly clonally reproducing *Usnea subfloridana* at the landscape scale, focusing on the comparison of lichen populations within hemiboreal forest stands. Particular attention has been paid to the genetic differentiation of lichen populations in two geographically distinct regions in Estonia and the relationships between forest characteristics and measures of genetic diversity. We genotyped 578 *Usnea* thalli from eleven lichen populations using seven specific fungal microsatellite markers. Measures of genetic diversity (allelic richness, Shannon's information index, Nei's unbiased genetic diversity, clonal diversity, the number of multilocus genotypes, the number of private alleles, and the minimum number of colonization events) were calculated and compared between *Usnea* populations. Shared haplotypes, gene flow and AMOVA analyses suggest that unconstrained gene flow and exchange of multilocus genotypes exist between the two geographically remote regions in Estonia. Stand age, mean circumference of the host tree, size of forest site and tree species composition did not show any significant influence on allelic richness, Shannon's information index, Nei's unbiased genetic diversity, clonal diversity, the number of private alleles, and the minimum number of colonization events of *U. subfloridana* populations. Therefore it was concluded that other factors of habitat heterogeneity could probably have a more significant effect on population genetics of *U. subfloridana* populations.

1. Introduction

The genetic diversity, an important part of overall biodiversity, enables evolutionary processes, which provide the raw material for adaptation to changing environments, and ensure healthy populations (Helm et al., 2009; Frankham et al., 2010). The genetic diversity of natural populations results from cumulative effects of historical and present-day processes (Hewitt, 2000; Frankham et al., 2010); the latter include, for example, changes in the current habitat conditions of the environment, which may influence dispersal, growth and vitality of species. Estimating the genetic variability within and among populations, and revealing genetic patterns of populations, improves out understanding of the population history, genetic differentiation and gene flow among populations (Werth et al., 2015). Population genetics also contributes to our knowledge of evolutionary processes, ecology, and conservation biology; for example, knowledge of genetic structure and variation of natural populations could be helpful in predicting the population fate in fluctuating environment (e.g., climate change or forest management) or estimating the effective population size of populations (Scheidegger and Werth, 2009; Ouborg, 2009).

Previously published studies regarding genetic structure and diversity of lichen-forming fungi led to different conclusions for each species studied and scale of geographical distribution (e.g., Werth, 2010; Scheidegger et al., 2012; Alors et al., 2017). The genetic diversity of a lichen population could be shaped by different factors of

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environmental heterogeneity. Habitat quality, measured as age or diameter of the host tree, is one of the most important factors affecting the genetic patterns of lichen-forming fungi populations (Otálora et al., 2011; Scheidegger et al., 2012). For example, Jüriado et al. (2011) found a higher genetic diversity in populations of the *Lobaria pulmonaria* (L.) Hoffm. and more juvenile thalli in old-growth forests compared with managed forests and wooded meadows. Furthermore, different types of disturbance (Werth et al., 2006a), environmental and microclimatic factors (Nadyeina et al., 2014a; Otálora et al., 2015) could also be significant in explaining the genetic structure and the distribution of gene pools of lichen populations.

Microsatellites or simple sequence repeats (SSR) are considered the most promising markers for investigating the genetic variation and population structure of highly clonal organisms such as lichens (Werth, 2010). The microsatellites are highly polymorphic, species-specific, and selectively neutral markers with a high mutation rate, which provides a more powerful resolution for estimating genetic diversity and variability among populations than former sequence-based method (Selkoe and Toonen, 2006; Werth, 2010). To date, microsatellite primers have been designed for several lichenized fungi (e.g., Prieto et al., 2015; Lutsak et al., 2016; Lagostina et al., 2017) and SSR markers have been successfully applied for studying the genetic diversity, phylogeographic structure, gene flow and genetic differentiation of lichen populations (e.g., Walser et al., 2003; Otálora et al., 2011; Nadyeina et al., 2014a). The majority of previous studies which have considered the population genetic variability of lichen-forming fungi using microsatellite markers have used the threatened, regionally rare or narrowly distributed lichens (e.g., Nadyeina et al., 2014a; Jones et al., 2015; Prieto et al., 2015), but only a few studies have reviewed the microsatellite diversity of common and widely distributed lichenized fungi and genetic structure of their populations (Mansournia et al., 2012; Degtjarenko et al., 2016; Alors et al., 2017).

In the current research we studied the population genetics of a common and widespread lichenized fungus at the landscape scale, focusing on a comparison of lichen populations within hemiboreal forest stands. To achieve this, the genetic variation at seven microsatellite loci in the mycobiont of the epiphytic lichen *Usnea subfloridana* Stirt. in Estonia, Northern Europe was investigated. The main objectives of this study were: (i) to study the genetic differentiation of *U. subfloridana* populations in two separate regions of Estonia; and (ii) to investigate the relationships between habitat characteristics and measures of genetic diversity of *U. subfloridana* populations.

2. Material and methods

2.1. Studied species

U. subfloridana is an epiphytic fruticose macrolichen with a wide distribution across Eurasia, Macaronesia, and North America (Nash et al., 2007; Randlane et al., 2009; Smith et al., 2009). It is very frequent and one of the most commonest *Usnea* species in Estonia, occurring mostly on Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*) and Silver birch (*Betula pendula*), and more rarely on other deciduous trees and lignum (Tõrra and Randlane, 2007; Randlane et al., 2011). *U. subfloridana* is not protected locally, and is red-listed in Estonia as Least Concerned (LC) (Randlane et al., 2008). This species reproduces asexually by symbiotic propagules, soralia and isidia, but could also propagate sexually, but specimens with apothecia are very rarely observed (Tõrra and Randlane, 2007; Randlane et al., 2011). Recent phylogenetic studies indicate that *U. subfloridana* is not a monophyletic entity but forms an intermixed clade with *Usnea florida* (L.) Weber ex F.H. Wigg., which is considered the primary, fertile counterpart of the sterile *U. subfloridana* (e.g., Articus et al., 2002; Saag et al., 2011; Mark et al., 2016). The apotheciate *U. florida* reproduces exclusively sexually and always lacks vegetative propagules; furthermore, it has distinct ecological requirements, preferring old deciduous trees in areas with a high atmospheric humidity (Randlane et al., 2009; Smith et al., 2009), while *U. subfloridana* is less ecologically demanding. To date, *U. florida* has not been recorded from Estonia (Tõrra and Randlane, 2007).

2.2. Study area

The study area is situated in two separate regions of Estonia, in Põlva County, in the southeastern region (hereafter SE), and in Lääne-Viru County, in the northern region (hereafter N) of Estonia, Northern Europe (Fig 1); the maximum distance between the two studied areas is 184 km. The study area has a characteristic temperate climate with a mean annual temperature of 6 °C; the mean annual precipitation is 672 mm, and the mean wind is 3.7 m/s (Estonian Weather Service, 2018). The vegetation of Estonia belongs to the hemiboreal forest zone, lying in the transitional area, where the southern taiga forest subzone changes into the spruce-hardwood subzone (Ahti et al., 1968; Laasimer and Masing, 1995). The two study sites (SE and N) are both located within the hemiboreal forest zone but in the different vegetation subdivisions, N Estonia being situated in the slightly oceanic to indifferent section, and SE Estonia in the indifferent to slightly continental section according (Ahti et al., 1968) and also in different regions according to the classifications based on sedimentary bedrock (Viiding, 1995) and soils (Reintam, 1962). The study was carried out in *P. sylvestris*-dominated boreal forests, belonging to the *Oxalis–Vaccinium myrtillus*, the *V. myrtillus*, and the *Vaccinium vitis-idaea* forest site types. These forest types are also widely distributed in other Baltic states (Kairiūkštis, 1966; Bušs, 1997), in Fennoscandia (Dierßen, 1996), and in northwest Russia (Fedorchuk et al., 2005).

2.3. Sampling

Fieldwork was carried out during the summer of 2011 (in SE Estonia) and the autumn of 2014 (in N Estonia). The potential localities for sampling were chosen from Forest Public Registry maps using comparable forest characteristics from their forest survey (Forest Public Registry, 2017). In total, *U. subfloridana* populations were sampled from eleven localities; eight populations from SE and three populations from N (Fig 1; Table 1). In each locality or lichen population, 30–62 samples were randomly collected from Norway spruce up to 6 m from the ground using a tree pruner (Table 1). On average, three *Usnea* thalli were taken from a host tree; if there were less than three thalli, only one or two specimens were sampled, while in other cases more than tree specimens were collected for balancing the sampling. *Usnea* populations were defined according to the boundaries of forest sites sharing the same values of forest survey data (forest site type, age of trees and proportion of trees in forest stand) according to Forest Public Registry (2017). The tree circumference (BHC) was recorded for each sampled tree at breast height (1.3 m). Other habitat characteristics (stand age, the proportion of pines and birches in forest stands, and size of forest site sharing the same values of data from forest survey) were provided by from Forest Public Registry (2017). Geographical coordinates were recorded per sampled tree with a GPS receiver Garmin GPSMAP 60C.

2.4. Chemical and molecular analyses

All collected *Usnea* thalli were air dried, cleaned to remove other lichen specimens, and examined under a stereomicroscope. Thin

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