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Small-scale analysis of population genetics and abundance patterns of honeysuckle *Lonicera periclymenum* L. (Caprifoliaceae) in a North Sea island woodland system

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Summary

Invasive alien plants are considered a major driving force of biodiversity loss and can deeply alter ecosystem functioning. However, invaders can also facilitate the distribution and establishment of native species, although this has been rarely documented in the literature. We observed an increase in population abundance of the liana *Lonicera periclymenum* L. (honeysuckle) on the north Frisian island of Amrum and hypothesized, that a surge in phorophyte availability due to the invasive neophyte *Prunus serotina* Ehrh. (black cherry) supported the colonization of new patches for this autochthonous liana species. Analysis of population genetics by inter-simple-sequence repeats polymerase chain reaction (ISSR-PCR) revealed high interpopulational differentiation. The genetic diversity within populations was low. The results indicated that barriers prevent small distance dispersal of seed and pollen. Further, additional results supported our hypothesis that honeysuckle may occasionally take advantage of the invasive neophyte black cherry.

Keywords: Caprifoliaceae, Invasive biology, island flora, biogeography, range expansion, North Sea, ISSR, *Prunus serotina*

Introduction

Patterns and processes of plant distribution and range expansion are strongly influenced by habitat changes. Silvicultural turnover in woodland areas, characterized by an intensive variability of structural properties due to deforestation and replanting provide ample space for changing floral composition of the forest habitat. One of the most striking effects under current studies are colonization processes by invasive taxa (e.g. RICHARDSON and REJMÁNEK, 2011; JUNG et al., 2017). However, autochthonous species may considerably profit from modifications in changing habitats of secondary origin, which eventually allow for reconnectivity of previously fragmented populations. In habitats where the floral composition of certain plant types is generally unsaturated, range expansion of either new invasive or native taxa is further enabled by anthropogenic changes in habitat structure (ODUOR, 2013). Earlier studies indicate that the relation of distance and dispersal ability of native woody and non-woody species from existing source populations influences recolonization patterns in secondary forest systems (DZWONKO, 1993; KADMON and PULLIAM, 1995; GRASHOF-BOKDAM, 1997; GRASHOF-BOKDAM et al., 1998).

Therefore, questions for our study can be raised about population structures of native woody plants in open dune-forest systems at the North Sea, which are naturally unsaturated for trees and shrubs and which are affected by forestal activities. Early settler cleared woodlands to create arable land and used the available wood to build houses until the late 19th century or even the early 20th century Amrum was naturally more or less free of trees (ERNST, 1934; FIRBAS, 1952), followed by two massive periods of planting in the late 19th century and mid-20th century (CHRISTIANSEN, 1961; RAABE, 1963; REMMERT, 1964; TÜRK, 1995; KOLLMANN, 1998). Due to the shortage of wood mainly conifers such as *Pinus sylvestris*, *Pinus mugo*, *Picea sitchensis* and *Pinus nigra* were planted (NAUMANN, 2010). However, pollen analyses show that a more natural vegetation on Amrum would be alders, birches, hazels, oaks and beeches (TÜRCK, 1995). In 1914 the woodland had already increased to about 26.5 ha for the regalement of spa guests. In 1962 the coastal woodland area reached its actual size of about 150 ha, that is about 10% of the islands area (KOLLMANN, 1998). Since the 1950s the predominanty coniferous woodland was successively replaced by mixed deciduous forest. Devastating hurricanes such as "Anatol" in 1999 or later "Kyrill" in 2007 led to the planting of a high level of native and non-native deciduous trees in the mixed stands. The destruction of the 1999 hurricane "Anatol" forced the inclusion of Amrum into the "Bergwaldprojekt". In addition, severe damage of Sitka spruce by the great spruce bark beetle *Dendroctonus micans* provided further space for reforestation in which already existing *Prunus serotina* Ehrh. (Rosaceae, black cherry) invaded.

Personal observations during the last decade gave the impression of an increase of *Lonicera periclymenum* L., a woody and native liana in the coastal forest system of the island of Amrum (North Sea), in distribution patches abundance. Honeysuckle is recorded from Amrum in all floras from 1930 on (GROOM, 2007) and regarded as a natural part of the native flora (TÜRK, 1994). We hypothesize, that the primary increase of phorophytes in the woodland system of Amrum, namely of the invasive *Prunus serotina* Ehrh. (Rosaceae, black cherry) (GROOM, 2007; JUNG et al., 2019), provide the physical space for the secondary spreading of *L. periclymenum*.

Therefore, we investigated colonization patterns and population diversity of *L. periclymenum* and compared it with an outgroup population at St. Peter-Ording (beeline 40 km). Additionally, we aim to obtain further insight in population biology of *L. periclymenum*, e.g. pollen and seed dispersal distances, by ISSR-PCR analysis of different *L. periclymenum* populations.

Materials and methods

Study area

To study the genetic diversity and differentiation of *Lonicera periclymenum* we sampled leave material on six sites at the edge of forests on Amrum, a north frisian island at the west coast of Germany. Material of an additional outgroup was collected on the mainland near St. Peter-Ording (beeline 40 km) (Tab. 1, Fig. 1).

Plant material was collected in the field in June 2017 and directly stored in silica gel. For the population genetic analyses a total of 70 individuals from seven populations (ten plants per population) in Schleswig-Holstein were investigated. Sampling sites 1 and 2 were located in dense woodland, sampling sites 3, 4 and 5 close to an unpaved path. Location 6 was separated from the main woodland by heathland. Sampling sites 1 to 6 were located on the island of Amrum and population 7 was located on the mainland near St. Peter-Ording. All distances between the populations in our study ranged from 1 km

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Tab. 1: Coordinates of sampling sites of *L. periclymenum* on Amrum and St. Peter-Ording and genetic diversity of *L. periclymenum* populations based on ISSR PCR. n = sample size used for genetic diversity, loc = number of loci scored, LocP = number of polymorphic loci, PLP (%) = percent polymorphic loci, Hj = expected heterozygosity, SE (Hj) = standard error of Hj.

Pop.	N	E	n	Loc	Loc P	PLP [%]	Hj	S.E. (HJ)
1	54°40´15.8	008°19´47.3	10	81	37	45.7	0.14649	0.01991
2	54°39´46.2	008°20´07.1	7	81	48	59.3	0.19301	0.01941
3	54°39´01.2	008°20´24.7	10	81	49	60,5	0.20492	0.02028
4	54°38´35.5	008°20´36.0	10	81	53	65.4	0.21455	0.01968
5	54°37´59.7	008°21´15.7	9	81	50	61.7	0.20949	0.02203
6	54°38´00.7	008°22´12.3	10	81	47	58.0	0.17463	0.01943
7	54°18´30.1	008°37´29.0	9	81	47	58.0	0.20065	0.02153

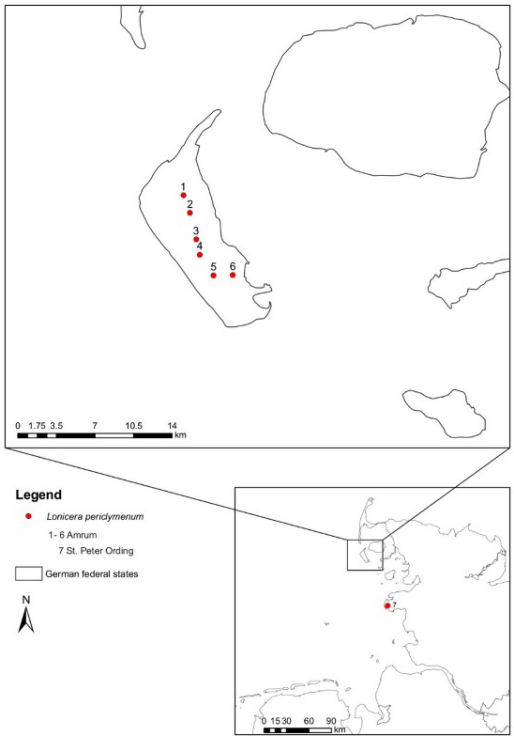

Fig. 1: Locations of sample sites of *L. periclymenum* on Amrum (1-6) and St. Peter-Ording (7). Map was prepared with ArcGIS Desktop (ArcGIS Desktop 10.2.2., Esri).

up to 2 km. To analyse the diversity of phorophytes, we determined all plants at the sampling sites and between the southern (site 6) and northern (site 1) populations on which *L. periclymenum* climbed.

Genetic analysis

Approximately 1 cm^2 dried plant material was shredded for DNA extraction using the DNeasy Plant mini Kit (Qiagen, Hilden, Germany) as described in the manufacturer's protocol. DNA quality and quantity were checked with a Nano Photometer (Implen, Munich) and was adjusted for further analysis to at least 10 ng/µl DNA per sample. To identify genetic differences between individuals inter-simple sequence repeats (ISSR) PCR was applied. This anonym marker technique utilizes single, microsatellite-complementary primers for the PCR and amplifies the regions between two microsatellites (Zietkiewicz et al., 1994; Ng and Tan, 2015). As these regions are not conserved and highly variable, ISSR-PCR is appropriate to detect genetic differences between individuals (Ng and Tan, 2015). In this case, 14 different ISSR primer were tested beforehand of which four produced suitable results and were chosen for this study. One primer was unanchored, two primers were 5´ anchored and one primer was 3´ anchored (Tab. 2) (Ng and Tan, 2015).

PCR was performed following a standard protocol with adapted annealing temperatures for each primer (Tab. 2). PCR mastermix contained 11 µl H_2O (Rotipuran Ultra, Roth), 2 µl 10× dream taq buffer including $MgCl_2$ (20 mM), 2 µl dNTPs (2 mM, Fermentas, Thermo Scientific), 0.4 µl single primer (10 pmol/µl, Metabion), 3 µl betain monohydrate (5 M, Sigma-Aldrich), 0.3 µl BSA (10 ng/µl, Fermentas, Thermo Scientific) and 0.3 µl Taq polymerase (5 U/µl, Fermentas, Thermo Scientific) for each sample. 1 µl DNA template was used for each PCR reaction. PCR started with an initial denaturation step for 3 min at 94 °C. Subsequently 35 amplification cycles were started including a denaturation step for 1 min at 94 °C, an annealing step for 0.5 min at primer specific temperature and an elongation step for 1 min at 72 °C. A final extension was performed for 10 min at 72 °C (Tab. 2).

Afterwards PCR products were separated applying agarose-gel electrophoresis. Therefore, products (12 µl) were mixed with SYBR Gold (3 µl) (Life Technologies, Darmstadt) and loaded on an agarose-gel (2.5%). To test for reproducibility, we used ten samples of different individuals as controls on each gel for every primer. Separation of the PCR products took place at 80 V for 150 min. For orientation the GeneRuler TM 100 bp Plus DNA Ladder (Fermentas, Life Sciences) was also added to the agarose-gel. Finally, amplified fragments were visualized under a UV transilluminator and photographed using a gel documentation system (Vilbert Lourmat Infinity model).

Tab. 2: Primers used for the ISSR analysis (Dogan et al., 2010).

Primer	Sequence	Annealing Temperature [°C]
F1	5`- GAG $(CAA)_5$ – 3`	49.1
F2	5`- CTC $(GT)_8$ – 3`	58.9
ISSR 4	5`- $(GACA)_3$ – 3`	38.5
M8	5`- $(AC)_9G$ – 3`	57

Data analysis

DNA- fragments were translated to a 0/1 matrix at which 0 encoded the absence of a fragment and 1 encoded the presence of a fragment at a particular gel position. Only clear bands were scored. Applying the Microsoft Excel add-in GenAlEx (Peakall and Smouse, 2012), several analyses were performed., We conducted each calculation with 999 permutations. An analysis of molecular variance (AMOVA) and a principal coordinate analysis were calculated to reveal genetic differentiation between populations. A Mantel test was performed to check for correlations between genetic differentiation and geographic distance (calculation was at individual level, using pairwise individual-by-individual genetic matrix and a corresponding geographic distance matrix). Genetic diversity within populations after Lynch and Milligan (1994) was calculated using AFLP surv (Vekemans

2002) with default options. The test for population genetic structure (Wright's fixation index, Fst after LYNCH and MILLIGAN (1994)) on 1% level was carried out with 500 permutations and 1000 bootstraps for genetic distances. In addition, the program SplitsTree4 (HUSON and BRYANT, 2006) was operated to generate a genetic network. Phenotypic error rate was calculated as number of phenotypic differences related to the total number of phenotypic comparisons and subsequently averaged over the four primer combinations (POMPANON et al., 2005; BONIN et al., 2004). The final overall error rate was under the 5% level (4.28%) (JONES et al., 1997; HANSEN et al., 1999).

Results

In total 81 loci were produced by the four primers, of which 78 (= 96.3%) were polymorphic. Population 1 has the lowest genetic diversity value (Hj = 0.14) and population 4 has the highest genetic diversity value (Hj = 0.21) (Tab. 1). AMOVA revealed a significant genetic differentiation of which more than a quarter is located among populations (28%, PhiP = 0.277, $p < 0.001$) (Tab. 3).

Principal coordinate analysis (PCoA) supports AMOVA and shows distinguishable grouping of the populations. Fig. 2A also shows eight outliers from the populations No. 2, 5 and 7 distorting the PCoA graph. Thus, Fig. 2B shows the PCoA graph without these outliers, illustrating that individuals from a population form more or less dense groups. Nonetheless, there is some intermingling of individuals from different populations and some populations are more differentiated than others are. Population 1 and 6 are the most confined populations and the populations with the lowest Hj values (Tab. 1). The phylogenetic network (Fig. 3) shows that individuals from the same population are more similar to each other than to individuals from other populations and as a result, it shows clustering of populations. The Mantel test revealed a significant positive correlation (Rxy = 0.227; $p > 0.011$; $R^2 = 0.0513$).

Surveying and mapping of the phorophytes revealed 26 species with a total number of 401 individuals. More than 20% of all investigated phorophytes were invasive black cherry (*Prunus serotina*), (Fig. 4). An almost similar proportion gained *Sorbus aucuparia* (16.71%). Other tree species with frequent presence of *L. periclymenum* were *Betula pendula* (10.97%) and *Alnus glutinosa* (9.23%).

Tab. 3: Genetic differentiation among and within populations.

Source of variation	PhiP	d.f.	Sums of square	Variation [%]
Among populations		6	286.157	28
Within populations		63	622.50	72
Total	0.277	69	908.657	100

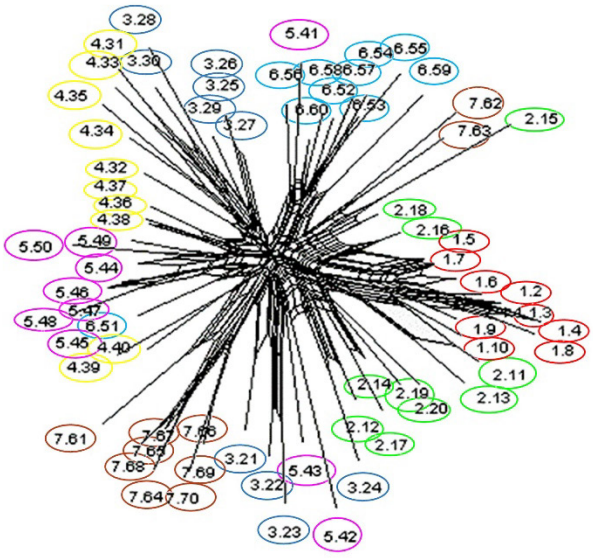

Fig. 3: Phylogenetic network (SplitsTree) of all individuals of *Lonicera periclymenum* on Amrum (Pop. 1-6) and St. Peter-Ording (Pop. 7), Schleswig-Holstein. Abbreviations of populations are indicated according to Tab. 1.

Discussion

Abundance and population structure of *Lonicera periclymenum* on Amrum evoke a number of questions concerning population diversity and ecological interaction. The high degree of genetic differentiation

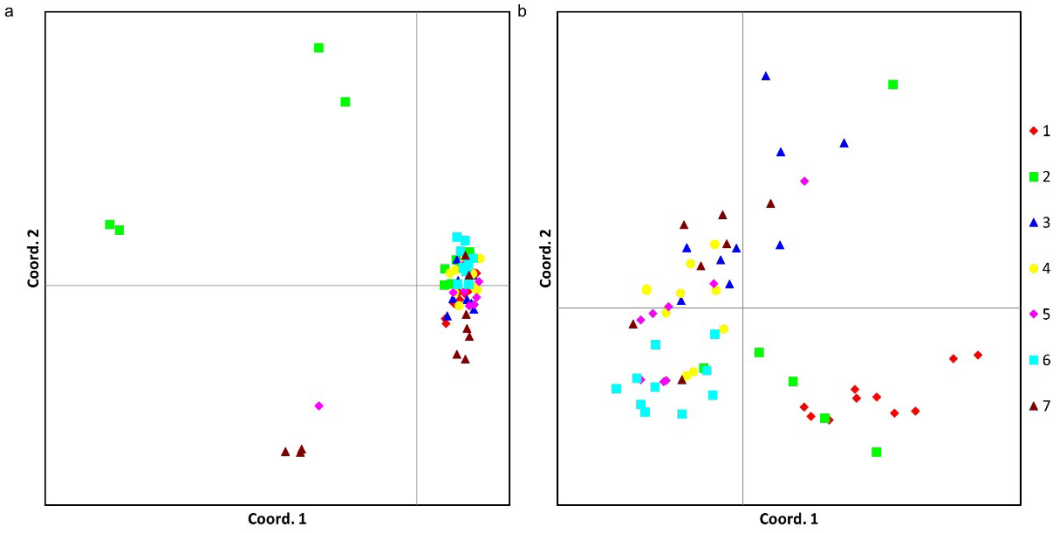

Fig. 2: Principal coordinate analysis (PCoA) for genetic distances between individuals of *Lonicera periclymenum* on Amrum (Pop. 1-6) and St. Peter-Ording (Pop. 7). a) including outlier. Total variation of Coord. 1: 31.38%, Coord. 2: 13.29% and Coord. 3: 8.41%. b) without outlier. Total variation of Coord. 1: 16.09%, Coord. 2: 13.46% and Coord. 3: 11.20%. Sampling regions indicated by colour and aberrations according to Tab. 1. Abbreviations of populations are indicated according to Tab. 1.

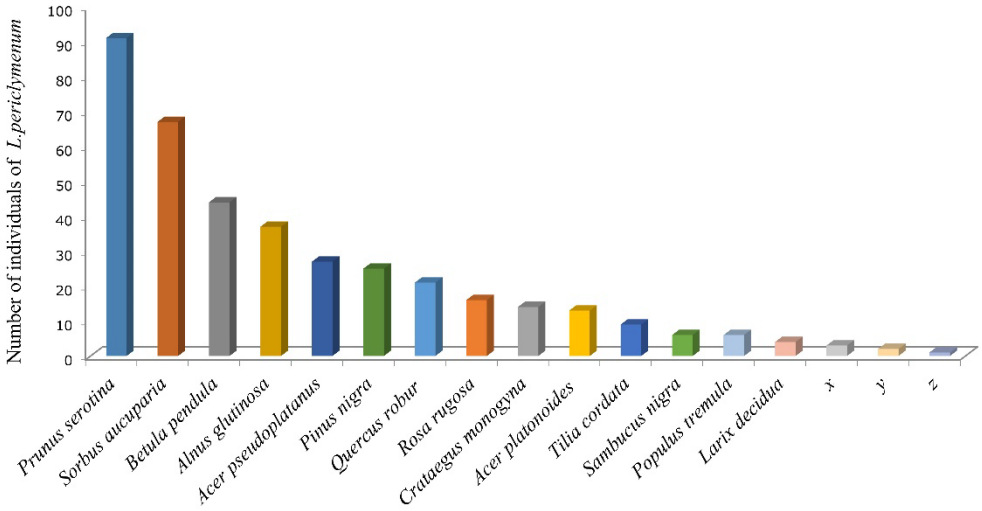

Fig. 4: Phorophytes of *Lonicera periclymenum* on Amrum. 26 species, 401 individuals in total. X: 3 individuals of *Fagus sylvatica*; *Quercus rubra*; *Salix caprea*. Y: 2 individuals of *Pinus silvestris; Prunus avium* each, Z: 1 individual of *Abies alba*; *Corylus avellana*; *Robinia pseudoacacia*; *Rosa spinosissima*; *Ulmus laevis* each.

between populations indicates the existence of barriers which impede genetic exchange. This on one hand might be pollinator driven, or on the other hand dispersal related.

Currently the investigated populations exhibit lower intrapopulational genetic diversity compared to interpopulational diversity. Grashof-Bokdam et al. (1998) already pointed towards limited gene flow between populations of *L. periclymenum* by an analysis of two woodlots using randomly amplified polymorphic DNA (RAPD). They found both: a) decreasing genetic similarity with geographical distance in a 300 m radius and b) genetic disconnectedness of populations. Grashof-Bokdam et al. (1998) determined the level for disconnectedness in their study to be a 2 km distance between populations. In our study area, this level for disconnectedness is even lower, since the distances between our populations were less than 2 km. The structure of the PCoA in Fig. 2 and the phylogenetic network in Fig. 3 are the result of a moderate disconnectedness between populations. Even below 2 km distance, populations are distinct from each other, explaining that there is no further separation of the mainland population from the island population.

Similarity within a population (300 m radius by Grashof-Bokdam et al., 1998) corresponds with the activity of pollinators. Ottosen (1987) observed male bumblebees of *Bombus hortorum* as the main pollinators for *L. periclymenum* and confirmed the length of their flight routes to be +/- 200 m. He also investigated types of pollination and concluded based on experiments with isolated flowers, that *L. periclymenum* follows a mixed pollination pathway. A significant part of seed production is by autogamy with about 65% seed set. Xenogamy was only observed in 20% of all pollination acts but yielded high seed set. Ottosen (1987) did not perform germination experiment of the seeds to test for viability, but concluded by the observation of numerous seedlings, that viability must be high. Grashof-Bokdam et al. (1998) concluded from their molecular analyses that seed dispersal of genetically similar seed (through autogamy) would count significantly for the homogeneity of the population structure. By use of the tetrazolium test, Guzzetti et al. (2017) determined the viability of ripe seeds of *L. periclymenum* to be 54%, and from their main phorophyte *P. serotina* even higher with 72%. These findings add a further proliferation potential apart from colonization via sucker formation to the invasive *P. serotina*, but also a significant potential to *L. periclymenum*, again apart from colonization via sucker formation.

However, in any case these barriers are related to woodland development and land use by silviculture. Population 1 and 6 are the most clearly differentiated populations. An explanation of this pattern could be that population 1 is located in a dense forest fragmented by a very small trail which could only be passed by pedestrians. Population 6 is located inside a small forest which is isolated from the large forest by surrounding heathlands. Accessibility for humans of these two populations is much lower than of the remaining populations. However, environment of population 2 was similar to the environment of population 1 but the genetic data of population 2 shows more outliers and are less united than genetic data of population 1. The remaining populations are much more accessible for pedestrians, cyclists and even small vehicles via large pathways. However, an indicative pattern of differentiation and direct human impact by accessibility could not be observed.

Most populations show some individuals, which are genetically different to the rest of the individuals belonging to the population. For example, the individuals 2.15, 2.16 and 2.18 do not group together with the remaining individuals of population 2 (Fig. 3). The same applies to the individuals 7.62 and 7.63. These outliers can be interpreted to be the result of occasionally long-distance dispersal of seeds by natural vectors such as birds or of plant material exchange during forestry (Pop. 2) and/or horticultural activities (Pop. 7). The location of Population 2 is one of the most disturbed woodland places on Amrum following the disturbances by Kyrill in 2007. Old coniferous and deciduous trees have been fully destroyed and cut in this place and massive natural regrowth of *P. serotina* from suckers has completely changed the flora in this area since 2007. Replanting activities of this area since 2016 changed the structure and abundance of phorophytes again but might also be responsible for the accidental introduction of *L. periclymenum* from tree nurseries, either from a different genotype of the wild species or from a cultivar. A similar effect could be the explanation for the outliers in Population 7 from the mainland. St. Peter-Ording has been shaped into a spa town for more than 150 years. Creation of hiking trails and planting of woodland for the regalement of spa guests changed the autochtonous floral composition into a horticulturally influenced flora. By this, cultivars of *L. periclymenum* might have been introduced and due to their longevity are still present (or reintroduced again from private gardens) in this area.

Our data shows that *L. periclymenum* utilizes *P. serotina* as a phorophyte, but other taxa such as *Sorbus aucuparia*, *Betula pendula* and *Alnus glutinosa*, all native to the Amrum Flora are also providing space for *L. periclymenum* to climb. In its native range *P. serotina* belongs to typical succession species and can germinate in

the absence of direct sunlight (McVaugh, 1951). However, for its further development *P. serotina* needs a sufficient amount of light and therefore loses its domination as soon as other species manage to overgrow it (Marquis, 1990). The still very abundant conifers on Amrum with their light canopy provide *P. serotina* with enough light to develop (Vanhellemant, 2009). Recent reforestation projects try to focus on more sustainable forest development and aim to establish mixed forests, which has no more the primary goal to provide wood but to be more natural. Therefore, mainly oak trees (*Quercus* spec.), birches (*Betula* spec.), linden (*Tilia* spec.), maple (*Acer* spe.) and copper beech (*Fagus sylvatica*) are planted, while *P. serotina* is regularly removed (Naumann, 2010). There is the possibility that these reforestation methods lead to a more natural forest on Amrum which will automatically repress the abundance of *P. serotina*. On the other hand, these efforts may fail and the forest on Amrum will stay anthropogenically disturbed. This will probably result in a further increase in *P. serotina* abundance. According to Oduor (2013) this will then have an intensive positive correlation between *L. periclymenum* and *P. serotina*, in which native *Lonicera* will be one of the plants which will profit from the spread of the invasive *Prunus*. It will be interesting to survey the spread and development of the genetic constitution of *L. periclymenum* populations in future.

The results support the idea of coexisting species whose fate significantly change by habitat changes due to silvicultural land use. In our system the neophyte *P. serotina* benefits on one hand from the naturally unsaturated wooden flora of Frisia, and on the other hand from secondary open space either from silviculture or from natural phenomena like the hurricanes "Anatol" in 1999 or "Kyrill" in 2007. The increase in numbers of *P. serotina* individuals is the base for the secondary spread of the autochtonous *L. periclymenum*, which benefits from the additional number of phorophytes, two sides of the medal "Invasiveness".

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Conflict of interest

No potential conflict of interest was reported by the authors.

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