Do landscape dissimilarity and environmental factors affect genetic and phenotypic variability in *Myosotis laxa* s. *lato* (Boraginaceae)?

Ene Kook*, Silvia Pihu, Ülle Reier, Marge Thetloff, Tsipe Aavik & Aveliina Helm

*Institute of Ecology and Earth Sciences, Tartu University, Lai 40, EE-51005 Tartu, Estonia* (*corresponding author’s e-mail: ene.kook@ut.ee*)

Received 2 Jan. 2015, final version received 11 Nov. 2015, accepted 11 Nov. 2015


*Myosotis laxa* s. *lato* (Boraginaceae) is a morphologically highly variable taxon. We examined whether, in the Baltic Sea region, the coastal form of *M. laxa* has a single centre of origin or if it has emerged due to landscape dissimilarity (sea/land ratio) and environmental factors independently in different regions. We used partial Mantel’s test to investigate correlations between the genetic and phenotypic variability of *M. laxa* and the mentioned habitat factors. Although the correlation between genetic distance and the sea/land ratio existed (*p* < 0.01), it was not strong (*r* = 0.34). Phenotypic distance among populations was not correlated with habitat factors. Similarly, there was no correlation between geographic distance and morphological characteristics of the studied populations. As neither phenotypic nor genetic dissimilarity between sampled locations was correlated with geographic distance, we suggest that the coastal form can arise independently in suitable habitats and its development may be caused by epigenetic regulation. However, gene flow among the coastal and mainland populations most likely prevents stronger adaptive and genetic divergence.

**Introduction**

A plant species can have different genotypes and phenotypes across its area of distribution, mainly due to geographic distance between populations (Still *et al.* 2005, Jenkins *et al.* 2010, Sexton *et al.* 2014). In addition, specific environmental conditions as well as landscape dissimilarity may significantly affect intraspecific phenotypic and genetic differentiation (Holderegger *et al.* 2010, Shafer & Wolf 2013, Sexton *et al.* 2014, Wang & Bradburd 2014). Intraspecific genetic differentiation in plants can increase due to adaptation to different environmental conditions, resulting in different locally-adapted genotypes (Temunovic *et al.* 2012, Baranzelli *et al.* 2014, Gray *et al.* 2014, Wang & Bradburd 2014). In addition to the direct effect of on-site environmental conditions, changes in the surrounding landscape can significantly affect gene flow among spatially-separated populations by enhancing or limiting pollen exchange and seed dispersal, and thereby

In addition to changes in adaptive genetic variation, plant phenotypes may respond to environmental conditions through phenotypic plasticity (Schlichting & Smith 2002, Valladares et al. 2007), or through epigenetic mechanisms that regulate gene expression under changing environmental conditions (Angers et al. 2010, Klironomos et al. 2013, Zhang et al. 2013). Thus, phenotypic plasticity and epigenetic regulation can prohibit or mask adaptive genetic changes inducing species’ response to environmental conditions without any change in adaptive genetic variation (Sultan & Spencer 2002, Chevin & Lande 2011, Scheiner & Holt 2012). For example, differences in the expression of the regulator genes between annual and perennial monocarpous life cycles are quite small, and a prolonged cold period in spring can lead to annual life cycles (Satake 2010).

Here, we studied the phenotypic and genetic variability of Myosotis laxa s. lato in the Baltic Sea region. Myosotis laxa s. lato is an octo-ploid herb in the family Boraginaceae, subfamily Cynoglosseae (Weigendt et al. 2010). The taxon is morphologically very variable (Grau & Merxmüller 1972, Apelgren 1990a, 1990b) and comprises annual, biennial and perennial life cycles (Grau & Merxmüller 1972, Ulvinaen 1998, Kühn et al. 2004, Koutecká & Lepš 2011, 2013). Based on the analysis of morphological traits, three subtaxa have been described in M. laxa s. lato. Individuals growing under the influence of brackish water in coastal habitats of the Baltic Sea typically differ from individuals growing on the mainland (Apelgren 1990b, 1990a, 1991). Coastal individuals of M. laxa are always annual, have leaves in the inflorescence, have longer pedicels and calyces, smaller flowers, and are shorter than individuals on the mainland (Samuelsson 1926, Grau & Merxmüller 1972, Krok & Almqvist 1994, Lazdauskaitë et al. 1996, Tzvelev 2000). Based on the distinctiveness of coastal individuals in the SW archipelago of Finland, Myosotis baltica was described by Samuelsson (1926) and later referred to as M. laxa ssp. baltica (Grau & Merxmüller 1972, Lazdauskaitë et al. 1996) or M. laxa var. baltica (Apelgren 1991, Krok & Almqvist 1994, Ulvinaen 1998). However, that taxon has recently been shown to be genetically indistinguishable from M. laxa ssp. caespitosa and coastal individuals should most likely be considered a variety or an ecotype rather than a subspecies (Pihl et al. 2009).

Myosotis laxa s. lato is widely distributed in Europe, Asia and North America (Grau & Merxmüller 1972, Hultén & Fries 1986), and according to BioFlor database (Kühn et al. 2004) across four floristic zones. The coastal form is restricted to the Baltic Sea region (Hultén & Fries 1986), and while most abundant in SW Finland and Åland (Ulvinaen 1998), it also occurs on the Estonian mainland and islands (Rebassoo 1960, Viljasoo 1969, Vissak 1991, Ploompui 1995, Rebassoo 1997, Kukk & Kull 2005). The origin and development of the coastal form is unknown. As the largest morphological variation within M. laxa s. lato occurs in the SW archipelago of Finland, it is speculated that the coastal form evolved there and later dispersed to other coastal regions around the Baltic Sea (Apelgren 1990b). However, the existence of M. laxa plants similar to the coastal form of M. laxa in the Caspian Basin, in the northern part of central Asia, Altai (Popov 1953, Viljasoo 1969) and Mongolia (Byazrov et al. 1983), casts doubt on a Finnish origin.

The post-glacial period in the Baltic Sea region is considered to have been too short for speciation (Ingelöö et al. 1993), but dynamic environmental conditions on the Baltic coast can lead to rapid population differentiation and the development of coastal microendemic species (Jonsell 1988). The coastal vegetation of the Baltic Sea is affected by post-glacial rebound, wave and ice erosion, annual water level fluctuations (low level in spring, high level in autumn) and temporal changes in soil salinity and pH (Ericson 1980, Jonsell 1988). As the Baltic Sea freezes in winter, spring is longer and occurs later in coastal areas than inland (Jaagus & Alas 2000).

Myosotis laxa s. lato is a poor competitor (Koutecká & Lepš 2011) and often grows on disturbed ground, where the surrounding vegetation is not closed (Jalas 1980, Koutecká
& Lepš 2011). Cessation of grazing probably decreases the abundance of *M. laxa* by reducing the number of suitable microhabitats. *Myosotis laxa* grows in different wet habitats, such as near fluvial and backwater, and in wet grasslands (BiolFlor, Kühn et al. 2004). It disperses via water (Fitter & Peat 1994). Changes in land use, as well as eutrophication and changes in landscape can impact small and temporary fluvial water bodies, thereby impeding the dispersal of *M. laxa*. According to von Numers (2011), *M. laxa* is decreasing in abundance and its distribution area in the SW archipelago of Finland is retreating northwards. These changes are occurring simultaneously with changes in land use (i.e. decrease of grazing; Kotiluoto 1998) and in environmental conditions (mostly eutrophication; Rönnberg & Bonsdorff 2004) in the coastal areas of SW Finland archipelago.

We focused on the coastal and mainland forms of *M. laxa* in the Baltic Sea region and investigated the effects of the sea/land ratio (a proxy for landscape dissimilarity) and environmental factors on their genetic and phenotypic differentiation. We expected environmental factors to be the main drivers of phenotypic variation. Although environmental factors may also have an effect on genetic variation (Holderegger et al. 2006), we assumed that the sea/land ratio is the main factor affecting the genetic variability of individuals within both coastal and mainland populations. The effect of geographic distance on genetic and phenotypic differentiation is also analysed to test the assumption that the coastal form of *M. laxa* originates from the SW Finland archipelago. If true, geographic distance would be a driver of both genetic and phenotypic variation and individuals from the area of origin would be expected to hold a basal position on the phylogenetic tree of *M. laxa s. lato*. On the other hand, an effect of the sea/land ratio or environmental factors on genotype or phenotype, would serve as evidence for an ecotypic origin of the coastal form of *M. laxa s. lato*.

**Material and methods**

**Data collection**

Specimens of *M. laxa* were sampled from 11 locations on the Estonian islands of Hiiumaa and Saaremaa, on the Estonian mainland, and on Hjortö and Björkö (Åland Islands, Finland) (Fig. 1). In total, 24 specimens were analysed. In three of the studied locations, both the coastal form and mainland forms were found. In those 'mixed populations', the mainland and coastal forms were considered separate populations, raising the final number studied of populations to 14. Specimens from Hiiumaa and Estonian mainland were previously studied by Pihu et al. (2009). The coastal form of *M. laxa* is very rare, and the number of populations as well as the number of individuals in the populations is very small. The samples selected for this study cover most of the known current localities of the coastal form in Estonia.
The proportion of land and sea within a 1-km radius around the populations were calculated based on Estonian base maps (Estonian Land Board, http://geoportaal.maaamet.ee [in Estonian]) and Finnish base maps (National Land Survey of Finland, http://www.maanmittauslaitos.fi [in Finnish]). Because water is considered to be the main seed dispersal vector for *M. laxa*, the sea/land ratio was used to describe the dissimilarity in landscape among populations. In addition, a number of environmental factors known to be important to plant performance were described for each sampling site. Information on soil moisture (dry or wet) and the effect of seawater (i.e. whether or not the sampled sites were periodically inundated) were obtained on site. Soil type was determined from the detailed soil map of Estonia (Estonian Land Board, http://geoportaal.maaamet.ee [in Estonian]) and the soil map of Finland (Geological Survey of Finland, http://geomaps2.gtk.fi/geo/ [in Finnish]). Precipitation during the driest and wettest months as well as minimum temperature in February (averaged over 1971–2000) were obtained from the Estonian Environmental Agency (http://ilmateenistus.ee) and the Finnish Meteorological Institute (http://en.ilmatieteenlaitos.fi). The environmental factors were pooled to calculate the environmental distance among populations.

Eleven morphological traits were measured from each sampled individual (Table 1) and the leaf samples were taken and dried in silica gel. These traits were previously used to distinguish different subtaxa of *M. laxa s. lato* (Pihu et al. 2009). The subtaxa (subsequently named coastal form and mainland form) were distinguished as in Pihu et al. (2009). Voucher specimens are stored in the herbarium of the Natural History Museum of the University of Tartu (TU).

### Genetic analysis

Genetic characteristics of the studied individuals were obtained from ITS1-5.8S-ITS2 sequences because this marker is expected to be variable both at intraspecific and interspecific levels (Mader et al. 2010, Poczai & Hyvönen 2010, Gao et al. 2012). DNA was extracted from silica-gel-dried leaf tissue samples using the standard protocol (Doyle & Doyle 1987), the ITS1-5.8S-ITS2 sequence was amplified with the primers ITS Leu1 and ITS4 (White et al. 1990). Polymerase chain reaction (PCR) was performed in a total volume of 20 μl containing 1x PCR buffer (Fermentas), 4 mM MgCl₂, 0.25M dNTP, 1 U of Smart-Taq DNA polymerase (Naxo), 0.8 pmol of each primer (ITS Leu1 and ITS4) (White et al. 1990, Vargas et al. 1999), 0.001 mg BSA (Fermentas) and 1–10 ng of template DNA.

Amplifications were performed as follows: 95 °C for 5 min and then 25 cycles of 94 °C for 30 sec, 53 °C for 30 sec and 72 °C for 2 min, final holding at 72 °C for 10 min. Nucleotide sequencing of PCR products was carried out on a 3730xl DNA Analyzer (Applied Biosystems) using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer’s protocol. Sequences were aligned in the BioEdit (Hall 1999) and corrected manually.

### Table 1. Measures of morphological variables and their trait states. Accuracy of metric measurements is ±0.5 mm.

<table>
<thead>
<tr>
<th>Character</th>
<th>Character states</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Number of leaves in inflorescence</td>
<td>0–6</td>
</tr>
<tr>
<td>2. Inflorescence type</td>
<td>sparse, dense</td>
</tr>
<tr>
<td>3. Length of longest pedicel at fruiting stage</td>
<td>in mm</td>
</tr>
<tr>
<td>4. Length of calyx of flower with longest pedicel at fruiting stage</td>
<td>not branched, branched in upper part, branched all over, branched in lower part</td>
</tr>
<tr>
<td>5. Branching intensity</td>
<td>up to 1 mm, 1–2 mm, 2–4 mm</td>
</tr>
<tr>
<td>6. Diameter of stem at base</td>
<td>reddish, not reddish</td>
</tr>
<tr>
<td>7. Colour of lower part of stem</td>
<td>fresh, dried</td>
</tr>
<tr>
<td>08. Condition of rosette leaves at flowering stage</td>
<td>in mm</td>
</tr>
<tr>
<td>09. Average length of leaf (of 2 leaves)</td>
<td>present, absent</td>
</tr>
<tr>
<td>10. Average width of leaf (of 2 leaves)</td>
<td>in mm</td>
</tr>
<tr>
<td>11. Presence of runners</td>
<td></td>
</tr>
</tbody>
</table>
Data analysis

For every population with 2 or 3 specimens, consensus sequence of ITS1-8.8S-ITS2 was created with BioEdit (Hall 1999). To elucidate phylogenetic relations among the studied 14 populations, neighbour-joining analysis was performed with MEGA 5.1 (Tamura et al. 2011) using the Jukes-Cantor method with 1000 bootstrap replications. All positions with less than 95% site coverage were excluded. The GenBank sequences of Trigonotis amblyosepala JX976814, T. formosana JQ388519, Myosotis arvensis AY092908 and M. scorpioides EU594655 were added as outgroup. To describe genetic differences between coastal and mainland forms, the Jukes-Cantor distance matrix for ITS1-8.8S-ITS2 consensus sequences was computed with MEGA (Tamura et al. 2011).

To describe the 14 populations morphologically, morphological trait values of individuals were averaged. For measurable traits, arithmetical means were computed (traits 3, 4, 9, 10; Table 1). For categorical traits, the most common state was selected for every population. If there were two different states in the population, the state most common in the whole data set was selected.

All other analyses were carried out in R (R Core Team 2013). The distance matrix of Gower distances compiling all morphological trait values for the sampled populations was compiled with the dist function in the stats package. To describe dissimilarity in the surrounding landscape and between environmental conditions in the populations, distance matrices of landscape and environment were computed with the vegdist function in the vegan package (Gower distances). Geographical distance matrix was computed with the earth.dist function in the fossil package.

To investigate correlations between the pairs of distance matrices described above, we performed partial Mantel's test using the mantel. partial function in the vegan package (Pearson method, 10 000 permutations). Correlations were calculated for the following pairs of matrices, all controlled for geographic distance as a third (z) parameter: (1) sea/land ratio and genetic distances, (2) sea/land ratio and phenotypic distances, (3) environmental dissimilarity and genetic distances, and (4) environmental dissimilarity and phenotypic distances. The correlation between genetic and phenotypic distances, and their correlation with geographic distance was tested with simple Mantel's test using the mantel function in the vegan package (Pearson method, 10 000 permutations).

Results

On the neighbour-joining tree based on consensus sequences of the 14 populations, M. scorpioides and M. laxa s. lato were connected in a clade (bootstrap support 100), and all populations of M. laxa s. lato clustered together with bootstrap support 75 (Fig. 2). Three subclades of the M. laxa clade had significant bootstrap support (Fig. 2; bootstrap support 54, 82 and 67). Clustering into subclades was not concordant with geographic origin of populations and coastal, mainland and mixed populations of M. laxa s. lato had mixed positioning within the subclades. Populations from Åland did not hold a basal position. Genetic distances in the populations were similar to interpopulation distances (Table 2).

Although the correlation (Mantel's test) between genetic distance and the sea/land ratio existed ($p < 0.01$), it was not strong ($r = 0.34$, Table 3). Phenotypic distance was related to neither the sea/land ratio nor environmental dissimilarity among populations (Table 3). Geographic distance alone had no significant effect on genetic or phenotypic variation (Table 3). A correlation between genetic and phenotypic distances was marginal ($r = 0.2$, $p = 0.03$).

Discussion

As a result of extensive landscape and climate changes during the last century, the interplay between genetic and environmental factors, ecotypic divergence and gene flow has been the focus of various recent studies (e.g. Ellmer et al. 2011, Andrew et al. 2012, Buehler et al. 2012, Shafer & Wolf 2013). In the present study, correlations of genetic distances among the populations of M. laxa with phenotypic distances and
the sea/land ratios were not strong. Environmental dissimilarity was not correlated with genetic or phenotypic distance. Based on our results, we conclude that gene flow from the mainland populations to the coastal populations and epigenetic regulation of life cycle possibly hinder genetic differentiation and evolution of special ‘coastal’ genotype in the *M. laxa* populations. As geographic distance had no effect on genetic or phenotypic distance, we suggest that the coastal form has no single centre of origin.

On the neighbour-joining tree based on the consensus sequences of *M. laxa* populations, all *M. laxa* individuals clustered together (bootstrap support 75) with *M. scorpionoides* as sister taxon (Fig. 2). Subclusters of *M. laxa* had significant bootstrap support but the clustering corresponded to neither geographic location nor coastal/mainland form. Because coastal and mainland form did not differ genetically according to individual-based neighbour-joining analysis (data not shown), there seems to be no special ‘coastal’ genotype. Likely, there are

### Table 2. Genetic distances within and between coastal and mainland form of *Myosots laxa s. lato* (computed with Mega 5.1, Jukes-Cantor model); ‘mixed coastal’ consists of all coastal individuals from mixed populations and ‘mixed mainland’ consists all mainland individuals from mixed populations.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 coastal</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 mainland</td>
<td>0.002</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 mixed coastal</td>
<td>0.002</td>
<td>0.003</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>4 mixed mainland</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
<td>0.005</td>
</tr>
</tbody>
</table>

### Table 3. Effect of landscape (sea/land ratio) and environmental dissimilarity on genetic and phenotypic distance between populations (*n* = 14) of *Myosots laxa s. lato*. Partial Mantel’s test was used to control for the effect of the geographic distance. The last column shows the sole effect (regular Mantel’s test) of geographic distance on genetic and phenotypic distance. Existing correlation is indicated with boldface.

<table>
<thead>
<tr>
<th></th>
<th>Sea/land ratio</th>
<th>Environmental dissimilarity</th>
<th>Geographic distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic distance</td>
<td><em>r = 0.34, p &lt; 0.01</em></td>
<td><em>r = 0.09, p = 0.24</em></td>
<td><em>r = −0.03, p = 0.59</em></td>
</tr>
<tr>
<td>Phenotypic distance</td>
<td><em>r = 0.17, p = 0.08</em></td>
<td><em>r = −0.11, p = 0.77</em></td>
<td><em>r = −0.1, p = 0.72</em></td>
</tr>
</tbody>
</table>
slightly different genotypes in _M. laxa_, which are all able to express both mainland and coastal phenotype. This finding is in accordance with the findings that plants can inhabit very different habitats without expressing broad genetic variability or different adaptive genotypes of the species (Richards et al. 2012, Rollins et al. 2013).

An effect of surrounding landscape (sea/land ratio) on the genetic distance of _M. laxa_ populations found in this study was not strong (Table 3). Composition of surrounding landscape is related to seed dispersal and gene flow (Bullock et al. 2001, Sork & Smouse 2006). It is likely that seed dispersal of _M. laxa_ occurs mostly unidirectionally from larger and more stable populations on the mainland to more dynamic and disturbed coastal habitats. This assumption fits the counter-gradient gene flow scenario (Sexton et al. 2014), which should be considered if gene flow is directed by environmental factors or greater propagule production in central populations (Endler 1973, Barton 2001). Greater seed production of the mainland form of _M. laxa_ is plausible because the plants of mainland form are larger, more branched, have more flowers and a longer flowering time than the coastal form (Samuelsson 1926, Viljasoo 1969, Krok & Almquist 1994, Ulvinen 1998, Tzvelev 2000). Dispersal of _M. laxa_ by water is likely to promote unidirectional gene flow from higher mainland populations to sea-level coastal populations. Possible consequences of counter-gradient gene flow are inhibited genetic differentiation in the populations (Sexton et al. 2014) and selection for plasticity and epigenetic effects (Sultan & Spencer 2002, Scheiner & Holt 2012). We found no significant correlation between environmental factors and genetic distances of _M. laxa_, suggesting no isolation by environment (IBE, reviewed by Shafer & Wolf 2013, Sexton et al. 2014, Wang & Bradburd 2014). Lack of IBE is not very common in plants, but it may be related to the occurrence of directed gene flow (Sexton et al. 2014). Our dataset also contained some coastal populations, in which coastal and mainland forms grew together. This may be considered an indirect evidence of a continuous distribution towards the sea followed by adaptation to environmental conditions of the coast. Co-occurrence of the two forms in coastal conditions can indicate that at least the first generations of mainland form are able to grow on coasts without manifesting any morphological response. However, the coastal environmental is rather harsh, and likely only a fraction of individuals with particular traits have a higher likelihood of longer persistence. Populations on the coast of the Baltic Sea are highly likely to suffer under the impact of water level fluctuations, wave and ice erosion or submergence by seaweed piles after storms, leading to higher susceptibility of local extinctions of populations. Across its distribution area, the coastal form of _M. laxa_ is less abundant than the mainland form (Grau & Merxmüller 1972, Jalas 1980, Hultén & Fries 1986, Krok & Almquist 1994, Lazdauskaitė et al. 1996, Ulvinen 1998, Kukk & Kull 2005). We speculate that coastal populations can die off and recover again from time to time due to the sink and source dynamics between coastal and mainland populations (sensu Eriksson 1996). As there was no significant genetic difference among the studied populations of coastal and mainland forms, it is likely that gene flow can limit adaptive genetic divergence in the populations of _M. laxa_.

Phenotypic distance of _M. laxa_ was not correlated with environmental dissimilarity. Although it is expected that the environment has a significant impact on phenotype, there were three mixed populations in our study containing both mainland and coastal forms and exhibiting different phenotypes in exactly the same environmental conditions. It is likely that phenotypic traits of the coastal form are developing not as a direct response to the environmental conditions on the coast, but as a result of an epigenetically regulated shift in the life cycle from biennial/perennial to annual. Differences in the phenotype of coastal individuals as compared with the mainland form — smaller size, presence of leaves in inflorescence, bigger seeds, and annual life cycle — can indicate adaptation to the shorter growing period on the coast. This kind of variation in life cycle is epigenetically controlled by _FLC_ (flowering locus C), in which prolonged cold periods can induce an annual life cycle (Aikawa et al. 2010, Satake 2010). The vegetation period starts later (the cold period is longer) in the coastal areas of Estonia than on the mainland due to the impact of cold surface waters of the Baltic sea in spring (Jaagus & Ahas 2000), thereby allow-
ing for cold-dependent epigenetic regulation of the life cycle of coastal plants. In addition, high water levels in late summer or autumn can be detrimental to plants that germinate in autumn (Jonsell 1988), therefore a short and rapid life cycle of annuals is more beneficial in the coastal areas of the Baltic Sea. *Myosotis laxa* plants similar to coastal form have also been found on river floodplains in Mongolia (Byazrov et al. 1983, see also http://greif.umi-greifswald.de/ floragreif/), with a similar effect caused by prolonged cold period and delayed spring.

The occurrence of plastic response (in a strict sense) to environmental conditions is another possible explanation for the occurrence of different phenotypes (Schlichting & Smith 2002, Crispo 2008). Plasticity is sustained by polyploidy (Hahn et al. 2012). The octoploid *M. laxa s. lato* (Ulvinen 1998) is expected to exhibit plastic response to environmental conditions. At the same time, plastic response to high water level is unlikely, as the annual high water level occurs typically in late summer or autumn (Ericson 1980, Sammelsson & Stigebrandt 1996), resulting in eradication of *M. laxa* adult plants and leaf rosettes that have not yet completed their life cycle.

Genetic distance and phenotypic distance of *M. laxa* were not correlated with geographic distance. Absence of correlation between genetic distance and geographic distance shows that phenotypically different populations of *M. laxa* belonging to the proposed subspecies *M. laxa ssp. baltica* have no single centre of origin.

Our results indicate that differences in the phenotype within *M. laxa* may at least partially have a genetic background, but are more likely caused by epigenetic response of plants to the environmental conditions in the habitat.

**Acknowledgements**

We are grateful to Carl-Adam Haeggström for helping with the fieldwork, Robert Szava-Kovats for proofreading and Meelis Pärts for valuable comments. We are thankful to anonymous reviewers, whose comments improved the article. This study was supported by the Estonian Research Council (grant no. 9223 for AH), the Estonian Ministry of Education and Research (IUT20-29), the Estonian Research Council (grant PUT589), and the EU through the European Regional Development Fund (Centre of Excellence FIBIR).

**References**


Ellmer M., Prentice H.C. & Andersson S. 2011: The struc-


Koutté E. & Lepš J. 2011: Performance of three closely related *Myosotis* species in an experiment in which substrate quality and competition were manipulated. — Preslia 83: 403–420.


Soviet Union], vol. 19: 366–368. Institute of Botany, Scientific Academy of Soviet Union, Leningrad. [In Russian].


Appendix. Environmental conditions of the studied populations. Soil types are classified according international standard for soil classification, provided by World Reference Base (WRB 2014).

<table>
<thead>
<tr>
<th>Population</th>
<th>Soil moisture</th>
<th>Minimum temperature (February, °C)</th>
<th>Average precipitation of the wettest month (mm)</th>
<th>Average precipitation of the driest month (mm)</th>
<th>Soil type</th>
<th>Impact of seawater</th>
</tr>
</thead>
<tbody>
<tr>
<td>coast_Läätsa_1</td>
<td>dry</td>
<td>−17</td>
<td>67</td>
<td>26</td>
<td>Calcaric Cambisol (K)</td>
<td>none</td>
</tr>
<tr>
<td>main_Läätsa_2</td>
<td>moist</td>
<td>−17</td>
<td>67</td>
<td>26</td>
<td>Arenosol (Arg)</td>
<td>none</td>
</tr>
<tr>
<td>coast_Rohuküla</td>
<td>moist</td>
<td>−19.1</td>
<td>83</td>
<td>34</td>
<td>Calcaric Cambisol (K)</td>
<td>present</td>
</tr>
<tr>
<td>coast_Sarve</td>
<td>moist</td>
<td>−14.9</td>
<td>83</td>
<td>37</td>
<td>Rendzic Leptosol (Kh)</td>
<td>present</td>
</tr>
<tr>
<td>main_Sarve</td>
<td>moist</td>
<td>−14.9</td>
<td>83</td>
<td>37</td>
<td>Rendzic Leptosol (Kh)</td>
<td>present</td>
</tr>
<tr>
<td>coast_Aland2</td>
<td>moist</td>
<td>−8.4</td>
<td>66</td>
<td>21.2</td>
<td>Gleysol (Lkg)</td>
<td>present</td>
</tr>
<tr>
<td>coast_Luidja</td>
<td>dry</td>
<td>−14.9</td>
<td>61</td>
<td>37</td>
<td>Gleysol (Lkg)</td>
<td>none</td>
</tr>
<tr>
<td>main_Aland1</td>
<td>moist</td>
<td>−8.4</td>
<td>66</td>
<td>21.2</td>
<td>Fluvisol (AG)</td>
<td>present</td>
</tr>
<tr>
<td>coast_Lihula</td>
<td>moist</td>
<td>−19.7</td>
<td>83</td>
<td>37</td>
<td>Gleysol (Lkg)</td>
<td>none</td>
</tr>
<tr>
<td>coast_Jausa1</td>
<td>moist</td>
<td>−14.9</td>
<td>61</td>
<td>37</td>
<td>Fluvisol (AG)</td>
<td>none</td>
</tr>
<tr>
<td>main_Jausa2</td>
<td>dry</td>
<td>−14.9</td>
<td>61</td>
<td>37</td>
<td>Calcaric Cambisol (K)</td>
<td>none</td>
</tr>
<tr>
<td>coast_Tiharu</td>
<td>moist</td>
<td>−14.9</td>
<td>61</td>
<td>37</td>
<td>Calcaric Cambisol (K)</td>
<td>present</td>
</tr>
<tr>
<td>main_Tiharu</td>
<td>moist</td>
<td>−14.9</td>
<td>61</td>
<td>37</td>
<td>Calcaric Cambisol (K)</td>
<td>present</td>
</tr>
<tr>
<td>main_Jausa1</td>
<td>moist</td>
<td>−14.9</td>
<td>61</td>
<td>37</td>
<td>Fluvisol (AG)</td>
<td>none</td>
</tr>
</tbody>
</table>