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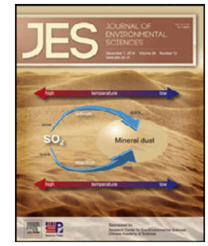

Persistent pollutants and the patchiness of urban green areas as drivers of genetic richness in the epiphytic moss *Leptodon smithii*

Valeria Spagnuolo[1], *Flavia De Nicola*[2,*], *Stefano Terracciano*[1], *Roberto Bargagli*[3], *Daniela Baldantoni*[4], *Fabrizio Monaci*[3], *Anna Alfani*[4], *Simonetta Giordano*[1]

1. *Department of Biology, University of Naples Federico II, via Cinthia 4, Naples 80126, Italy. E-mail:* vspagnuo@unina.it
2. *Department of Science and Technology, University of Sannio, Via Port'Arsa 11, Benevento 82100, Italy*
3. *Department of Environmental Sciences, University of Siena, Via Mattioli 4, Siena 53100, Italy*
4. *Department of Chemistry and Biology, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, SA, Italy*

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ABSTRACT

We determined genetic variation and metal and polycyclic aromatic hydrocarbon concentrations in *Leptodon smithii* moss collected in holm oak stands at cities, outskirts and remote areas of Campania and Tuscany (Italy) to investigate if anthropogenic pressure (pollutant emissions and land use change) affects moss genetic richness. In both regions, metal and polycyclic aromatic hydrocarbon concentrations reflected the trend urban > outskirts > remote areas, excepting Tuscany remote site. In both regions, the moss gene diversity increased from urban to remote areas. The findings suggest the extent and the fragmentation of urban green areas, as drivers of moss genetic richness.

Introduction

Urbanisation is an important driver of global land transformations. The progressive conversion of lands from wild and agricultural to urban and suburban areas determines habitat loss and fragmentation, environmental pollution, soil compaction and paving and changes in local climate (the "urban heat island"; Oke, 1995), with detrimental effects on biological diversity. The species occurring in urban landscapes are the combination of those colonizing novel habitats formed by urbanisation and those remaining after local extinction. As a rule, the management of urban landscapes, the environmental pollution and the extent and connectivity of urban green areas are deemed as the main factors affecting the composition/richness of species and their genetic variability in urban ecosystems. Organisms with high dispersal capability often exhibit a lower decline from rural to urban areas. Thus, communities may show a large similarity degree among cities, known as the "biotic homogenization" (Mc Kinney, 2006). This effect is usually ascribed to the common characteristics of urban environments regardless of the surrounding biome and to the transportation of similar subsets of species across geographical boundaries. Detecting the likely origin of species living in urban ecosystems will allow us to infer the broad scale processes which have led to species richness and genetic diversity in urban ecosystems (Sattler et al., 2011).

Most moss and lichen species have an extraordinary dispersal capability and can colonize the Earth's remotest regions; they have no roots and waxy cuticles, they are largely dependent on atmospheric deposition for their metabolism and the different species show a different sensitivity to atmospheric phytotoxic pollutants (Bargagli, 1998; Sim-Sim et al., 2000; Spagnuolo et al., 2011). Thus,

* *Corresponding author*. E-mail: fdenicol@unisannio.it (Flavia De Nicola).

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based on these features, during the last century the biological diversity (mainly estimated as species richness) of cryptogam communities has been used as an indication of air quality in urban environments (Le Blanc and De Sloover, 1970). During the last decades the concentrations of some "conventional" phytotoxic pollutants, such as SO_2, are decreasing; however, other atmospheric pollutants, such as trace metals and polycyclic aromatic hydrocarbons (PAHs), are mostly present in urban environments, largely emitted by circulating vehicles, affecting the chemical composition, physiology, and molecular diversity of lichens and mosses. At present, airborne particulate matter, *e.g.*, $PM_{2.5}$ with associated metals and PAHs, is among the most harmful pollutants (EEA, 2011). Due to technical difficulties and high costs, only some of these pollutants are monitored by physico-chemical approaches. Thus, the analysis of native moss tissues can provide a time-integrated evaluation of atmospheric deposition of many persistent pollutants not measured by automatic devices, evidencing spatial differences and temporal trends (Giordano et al., 2010, 2013; De Nicola et al., 2013b; Harmens et al., 2013; Spagnuolo et al., 2013). However, to make reliable comparisons among pollutant concentrations in moss samples of the same species collected in different urban sites, analysed samples must be the same age (*i.e.*, exposure time), and must have the same growth rate and form of growth (Zechmeister et al., 2003; Aboal et al., 2010). Thus, assuming that in an urban area several environmental variables such as precipitation, temperature and radiation are rather homogenous, some authors suggest taking into account the intraspecific genetic variation in biomonitoring studies, because it may affect adaptation mechanisms to airborne pollutants (Boquete et al., 2013).

In bryophyte island populations most species are clones, *i.e.*, populations with high local abundance, and often show a minimum genetic variation; according to Cronberg (2002) this variation increases with the population age. However, moss cross-transplantation experiments between polluted and unpolluted sites (Tabors et al., 2004; Boquete et al., 2013) indicate that mosses continuously exposed to high deposition of heavy metals probably undergo a genotypic adaptive response. Therefore, the molecular diversity of mosses in urban ecosystems is probably affected by historical as well as recent events such as atmospheric pollution.

A previous study on the moss *Leptodon smithii* F. Weber and D. Mohr (Spagnuolo et al., 2007), a facultative epiphytic species growing on barks of *Quercus ilex* L. (a tree of the Mediterranean climax vegetation, very common also in many urban environments), showed intra-population genetic variations among samples collected in urban, extra-urban and remote areas in southern Italy. As a rule, the molecular diversity decreased along an ecological/environmental gradient, with the lowest values in the sites with the highest habitat disturbance and fragmentation. These results suggested that *L. smithii* spores probably have a limited dispersal range and the moss population age and size, together with the connectivity of urban green areas are the main drivers of its genetic diversity. A subsequent study (Spagnuolo et al., 2009) along the same gradient of anthropogenic pressure showed an inverse relationship between the moss genetic variation (given as Nei's gene diversity; Nei, 1987) and the total metal load in moss tissues. Thus, in urban environments the genetic diversity of epiphytic mosses seems to be affected by relatively recent and ongoing processes such as the atmospheric deposition of pollutants. It is known that bryophytes can develop ecotypic variants adapted to survive in polluted environments (Shaw, 1994).

In the framework of a wider project aimed at evaluating the role of urban vegetation as interceptor and accumulator of persistent atmospheric pollutants, we determined genetic variations and pollutant concentrations in populations of the epiphytic moss *L. smithii* collected in holm oak stands at urban areas, outskirts and remote areas in two Italian regions (Campania and Tuscany) with different climatic and environmental conditions (De Nicola et al., 2013b). In this article, we tested the hypothesis that anthropogenic pressure, evaluated as pollutant emissions and land use change, affects genetic richness in native moss populations.

1. Material and methods

1.1. Study areas

L. smithii is a pleurocarpous, facultative epiphytic moss, rather common on tree bark of humid and close woods in almost all continents (Nelson, 1973). In Italy this species grows on trunks of *Q. ilex* L., a common tree of Mediterranean woods, widely used in the landscaping of many Italian urban parks and gardens.

Moss samples were collected in six holm oak stands, three in Campania and three in Tuscany (Table 1): the urban environments of Naples (C-1) and Siena (T-1), the relative outskirts (C-2 and T-2) and two remote areas (C-3 and T-3). All sampling areas have a typical Mediterranean climate (slightly warmer and moister in Campania). The municipalities of Siena and Naples occupy the same area but have different population sizes (54,500 *vs.* 957,600 inhabitants respectively); moreover, the province of Naples, whose area is one third that of Siena (1171 *vs.* 3821 km^2) has a much higher number of industrial plants, motor vehicles and other anthropogenic sources of atmospheric pollutants. The urban sampling site C-1 was located in a wide and historical park that houses the former Bourbon Royal Palace and served as a hunting game reserve for the kings of Naples. It is isolated from agricultural and forest areas and is bordered by heavily trafficked roads; the dominant trees are holm oaks, chestnut trees, magnolias and elm trees. The holm oak stand of outskirts (C-2) was located in an ash ring crater (250 ha wide), a State Nature Reserve covered by dense forest vegetation and with small permanent water bodies which contribute to more favourable conditions for vegetation during summer. The C-3 remote site was in a *Q. ilex* forest located in a protected natural area at about 100 km from C-1. In Siena the urban site (T-1) was located in a private garden park with large holm oak trees, near a heavily trafficked road. This small urban park (Table 1) is connected to suburban agricultural and forest areas. The site T-2 was about 3.5 km far from Siena's city centre; it has minimal air pollution and anthropogenic disturbance and is surrounded by agricultural areas and holm oak forests. The remote site in Tuscany (T-3) was located in an ancient forest with low density of *Q. ilex* trees growing near the sea coast in very xeric conditions (yearly precipitations about 650 mm, with 5 months of water deficit; Bussotti et al., 2002).

1.2. Metal and PAH analyses

At each site, in spring 2009, small green clumps of the epiphytic moss *L. smithii* were collected from several holm oaks, at a height of 1–2 m above the ground. All clumps were carefully mixed to obtain a composite sample. At the basis of the same holm oak trees, after litter removing, surface soil subsamples (0–5 cm) were also collected by a plastic cylindrical corer and mixed to obtain a composite soil sample, in order to define the contribution of soil particles to the total metal concentrations in mosses.