Science of the Total Environment 640–641 (2018) 678–687

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Temperature and herbivory, but not pollution, affect fluctuating asymmetry of mountain birch leaves: Results of 25-year monitoring around the copper nickel smelter in Monchegorsk, northwestern Russia

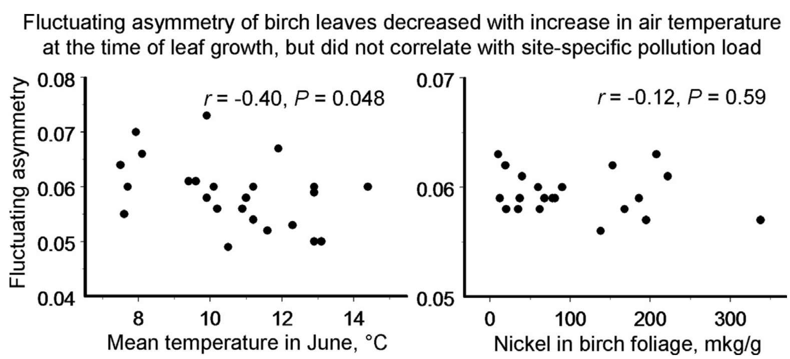
Mikhail V. Kozlov *, Vitali Zverev

Section of Ecology, Department of Biology, University of Turku, FI-20014 Turku, Finland

HIGHLIGHTS

- Fluctuating asymmetry (FA) is a commonly used indicator of environmental stress.
- Responses of leaf FA to different stressors were idiosyncratic in mountain birch.
- Leaf FA was independent of industrial pollution load in both space and time.
- Leaf FA increased with decrease in air temperature and with increase in herbivory.
- Leaf FA should be measured in a blinded manner to avoid confirmation bias.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 April 2018
Received in revised form 7 May 2018
Accepted 26 May 2018
Available online xxxx

Editor: Elena Paoletti

Keywords:
Background insect herbivory
Climate warming
Confirmation bias
Developmental stability
Ecological indicators
Industrial pollution

ABSTRACT

Fluctuating asymmetry (FA), which is defined as the magnitude of the random deviations from a symmetrical shape, reflects developmental instability and is commonly assumed to increase under environmental and genetic stress. We monitored the leaf FA of mountain birch, *Betula pubescens* subsp. *czerepanovii*, from 1993 to 2017 in individually marked trees at 21 sites around the copper nickel smelter at Monchegorsk, and we then analysed the results with respect to spatial and temporal variation in pollution, climate and background insect herbivory. Responses of leaf FA to different stressors were stressor specific: FA did not correlate with pollution load, it decreased significantly with an increase in June air temperature and it increased slightly but significantly with an increase in the previous-year leaf damage due to defoliating and leafmining insects. Our findings suggest that climate warming is unlikely to impose stress on the explored mountain birch populations, but even small increases in insect herbivory may adversely affect birch trees. However, these conclusions, since they are based on an observational study, should be viewed as tentative until confirmed by controlled experiments. We also demonstrated that the use of non-blinded measurements, which are prone to confirmation bias, was the primary reason for the earlier report of an increase in birch leaf FA near the Monchegorsk smelter. We hope that our findings will promote a wide use of blinded methods in ecological research and that they will contribute to debunking the myth that plant leaf FA consistently increases with increases in environmental pollution.

1. Introduction

Much of our knowledge about the state of ecological systems and their responses to various disturbances has originated from ecological

* Corresponding author.
E-mail address: mikoz@utu.fi (M.V. Kozlov).

https://doi.org/10.1016/j.scitotenv.2018.05.328
0048-9697/

monitoring. The development of the concept of forest monitoring started in 1970s, in particular due to concerns about potential transboundary pollution effects on forests, which gave the rise to several national and international monitoring programs (Ferretti, 2013). The importance of monitoring disturbances in ecosystem structure and functions is receiving increasing attention, driven by the current intensification of research into natural disturbances due to climate change (Thom and Seidl, 2016) and the emphasis on worldwide recovery of ecosystems from anthropogenic disturbances (Moreno-Mateos et al., 2017).

The existing forest monitoring programs, as a first line of investigation, serve practical goals because ecological management requires timely prediction of emerging environmental problems. This requirement gives special value to long-term data collected on ecological indicators (i.e. on those characteristics of organisms or ecosystems that could be used as early warning systems to signal imminent but not yet apparent problems; Dale and Beyeler, 2001). One such indicator, introduced in the early 1990s, is fluctuating asymmetry (FA), which is defined as small, non-directional deviations from perfect symmetry in morphological characters of plants and animals. FA has long been advertised as a universal and easy to measure stress indicator (Zakharov, 1990; Clarke, 1992; Parsons, 1992; Graham et al., 1993; Freeman et al., 1993). However, to our knowledge, none of the existing forest monitoring programs involves FA measurements in plants. Consequently, long-term data on plant FA, collected from the same study sites and the same plant individuals, are virtually non-existent.

Long ago, one of us (MVK) suggested that the FA of birch leaves could serve as a convenient indicator for rapid assessment of environmental pollution impacts on plant performance (Kozlov et al., 1996). Based on this hypothesis, in 1993, we established a local monitoring program in multiple sites around the copper nickel smelter in Monchegorsk in northwestern Russia. However, the results of the first 8 years (1993–2000) of monitoring of leaf FA of mountain birch, *Betula pubescens* subsp. *czerepanovii* (Orlova) Hämet-Ahti, surprisingly demonstrated no correlation between FA and heavy metal or sulphur dioxide concentrations around this smelter (Valkama and Kozlov, 2001). At the same time, the birch leaf FA appeared to increase with a decrease in ambient air temperature in June, the month when expansion of birch leaves occurs in our study region (Valkama and Kozlov, 2001). This observation was later supported by Hagen et al. (2008) who found higher leaf FA of mountain birch in northern Norway in the year with lower ambient air temperatures during the period of birch leaf growth.

Keeping in mind the substantial decline in emissions from the Monchegorsk smelter in 1990s and the rapid increase in early summer temperatures in 2000s (Zvereva et al., 2016), we continued to monitor birch leaf FA until 2017 in order to elucidate combined effects of pollution and climate on this potential indicator of environmental quality. Plant FA can also increase in response to herbivory (Zvereva et al., 1997a, 1997b; Møller and Shykoff, 1999; Kozlov, 2005), which, in turn, can be influenced by pollution (Zvereva and Kozlov, 2010). Therefore, starting from 1999, we also measured the percentages of leaves damaged by defoliating and leafmining insects in our monitoring trees by the end of the growth season (Kozlov et al., 2017).

Earlier, we found a significant variation in FA among individual birches within our study plots (Valkama and Kozlov, 2001). This variation suggested that birch genets either responded differently to environmental stress or they demonstrated different degrees of genetic stress. Plant FA increases with hybridisation (Wilsey et al., 1998; Albarrán-Lara et al., 2010), which makes the impact of birch genotype on environmentally induced changes in leaf FA very likely, as birches often form interspecific hybrids (Anamthawat-Jonsson and Thorsson, 2003; Koropachinskii, 2013). In particular, mountain birch is thought to have originated through introgressive hybridisation between the southern *B. pubescens* Ehrh. and the northern *B. nana* L. (Elkington, 1968; Vaarama and Valanne, 1973).

During the past decade, the initial optimism regarding studies of FA disappeared due to steady accumulation of inconclusive and negative results. A large number of field observations (reviewed by Graham et al., 2010; Kozlov, 2017) and controlled experiments (Roy and Stanton, 1999; Andalo et al., 2000; Sandner and Matthies, 2017; Zverev et al., 2018) have failed to detect the expected effects of stress on FA. Nevertheless, scientific questions remain regarding how environmental and genetic factors influence FA (Graham et al., 2010; Klingenberg, 2015), and this gave paramount importance to our uninterrupted, 25-year-long, time series, with data mostly collected from the same plant individuals.

In the present study, we report the spatial and temporal variations in leaf FA of mountain birch around the Monchegorsk smelter, and we explore factors that could explain this variation. We hypothesised that leaf FA: 1) increases with decreasing distance from the smelter due to an increase in pollution load and/or pollution-induced habitat disturbance; 2) decreases with the decreasing annual emission of pollutants due to partial alleviation of pollution-induced stress; 3) decreases during the years with increased ambient air temperature in June due to partial alleviation of climatic stress at the time of leaf expansion; 4) increases with increasing previous-year insect herbivory; and 5) differs among individual trees within the study site, presumably due to different genotypes and/or different sensitivity of individual genotypes to pollution and climatic stress. We tested our hypotheses 1–5 by analysing leaf FA values of 24,943 leaves collected during 1993–2017 from 195 mountain birches growing naturally at 21 sites located 1 to 64 km from the Monchegorsk smelter. We also: 6) suggested that imperfect research methodology, primarily the use of non-blinded measurements, contributed to the earlier conclusion (Kozlov et al., 1996) regarding significant increases in leaf FA of mountain birch near Monchegorsk, and we tested this hypothesis by re-measuring 600 leaves that had served as the basis for that original conclusion.

2. Materials and methods

2.1. Study area and study sites

The monitoring was conducted in the central part of the Kola Peninsula, which is located in the north-west of Russia, next to Finland and Norway, to the north of the Polar Circle (Fig. 1). The nickel–copper smelter in the town of Monchegorsk (67°56′ N, 32°49′ E), about 150 km south of the tree line, was one of the largest industrial polluters in the Northern hemisphere for decades. The smelter began production in 1937–1938 and had no air-cleaning facilities until 1968. The annual emissions of sulphur dioxide reached a maximum of 278,000 metric tons (t) in 1983, steadily decreased to about 100,000 t by the mid-1990s, dropped to 45,000 t in 1999 and have remained at about this level since then (Table S1). Metal emissions during the 1980s–1990s were 3000–8000 t of nickel and 1000–6000 t of copper annually and then declined in concert with declines in SO_2 (Table S1). For the history of pollution impacts on the study region and the levels of environmental degradation, consult Kozlov and Barcan (2000), Kozlov et al. (2009) and Manninen et al. (2015).

We monitored different characteristics of mature (over 20 years old) individuals of mountain birch growing naturally at 21 sites located in both pristine (unpolluted) subarctic Norway spruce (*Picea abies* (L.) Karst.) forests (Fig. S1) and in forests exhibiting different levels of pollution-induced deterioration (Fig. S2), including industrial barrens (Fig. S3), at distances 1 to 64 km from the smelter (Fig. 1; Table S2). The monitoring sites were selected along the main roads to allow easy access, but at least 50 m from the roadside to minimise impacts of traffic pollution. In 1993, we established 20 sites and individually tagged five haphazardly selected mature birches per site; 29 of these 100 birches were monitored until 2017. In 1996, we added one more site (5 N) to close the gap between the sites located 3 and 8 km north of the smelter; but this site was sampled uninterruptedly only from 2003. In the years