ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 55, nr 3: 517-527
1986

Gene flow in parapatric plant populations of *Agrostis tenuis* L. and *Festuca rubra* L.

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(Received: December 17, 1985. Accepted: February 6, 1986)

Abstract

The gene flow between populations of *Agrostis tenuis* L. and *Festuca rubra* L. on a Zn/Pb mine and adjacent areas, which met at a distance of 150 m from the mine boundaries, was studied. It was found that the mine populations exhibit a high index of tolerance to Zn and Pb, elements encountered in the soil. Those outside the mine show relatively high indices of tolerance towards Zn and Pb, despite the fact that these elements are absent from the soil. On the other hand, the index of tolerance towards Cu, absent from the soil, of populations inside and outside the mine, was very low and did not differ from that of the control. Time difference in the flowering of these parapatric populations acts against gene flow thus resulting in a tendency for the neighbouring populations to isolate.

Key words: Agrostis tenuis, Festuca rubra, Poaceae, gene flow, selection, zinc-lead tolerance

INTRODUCTION

The cause of restricted plant development on mine sites or old abandoned mines has long been the object of study for many researchers (Ashida 1965, Antonovics et al. 1971, Proctor and Woodell 1975, Foy et al. 1977, Bradshaw and Chadwick 1980).

Physical and chemical properties of the soil, lack of light and water are undoubtedly some of the major factors determining restriction of plant development. These soil peculiarities of mines or old abandoned ones are usually due to their chemical composition. Therefore, in contrast to primary development factors, edaphic differences mainly deriving from differences in the soil chemical composition, can very well be pinned down resulting in the occurence of characteristic areas of plant distribution.

However, there are some species among which tolerant ecotypes have evolved with the capacity to colonize contaminated areas of this sort in quite a satisfactory fashion (Bradshaw 1952, 1959, 1970, Jowett 1958, 1964, Antonovics et al. 1971, Karataglis 1978a, b, 1980, 1981, 1982).

It is possible for the populations of one and the same plant species to grow under entirely different edaphic conditions (Kruckeberg 1951, Gregory and Bradshaw 1965, Antonovics et al. 1971). Most of these natural populations are separated from each other by long distances or other physical barriers which prevent gene exchange and consequently remain morphologically and genotypically distinct (allopatric populations). They may also develop in adjacent regions (parapatric populations), in which case gene flow occurring between them happens to be so intense that these two natural populations display less distinct morphological and genotypical characteristics.

Gene flow has been observed both theoretically as well as evaluated on natural populations in the field (Karataglis 1980). As a result, several models have been established. Field measurements have shown that although pollen is likely to be carried for long distances by wind or insects, cross-fertilization mainly occurs at distances close to their natural source.

As regards field investigation, our knowledge on the extent of gene flow among neighbouring natural populations has been poor, particularly in parapatric populations whose gene flow is such that the distinction between these two adjacent populations becomes less evident. To this end some experiments were done wishing to examine:

1. The tolerance levels of *Agrostis tenuis* and *Festuca rubra* populations against heavy toxic metals (Zn, Pb and Cu) on the mine site, which, in their turn, are probably going to affect the adjacent populations.
2. The distribution of tolerance against these heavy metals along three transects, starting from the mine towards the neighbouring outside area.
3. The degree of gene flow occurring between populations, along transects and the probable influence on them by natural selection.
4. The flowering time of adjacent populations which is one of the major causes of population isolation.

MATERIAL AND METHODS

COLLECTION OF PLANT MATERIAL

On the mine site, *Agrostis tenuis* and *Festuca rubra* were the most dominant species, *Deschampsia caespitosa*, *Rumex acetosa*, *Plantago lanceolata* and a few others were more or less frequent.

A tenuis and *F. rubra* coexisted in several places inside and outside the mine. Five different positions randomly selected from the mine site gave us *A. tenuis* and *F. rubra* populations whose tolerance towards Zn, Pb and Cu was to be examined.

The rest of the collections were made along three transects starting from the inside area of the mine near its boundaries, passing on to the outside neighbouring site. The distance between the three transects of the parallel collections was about 10 m. As one can see, collections were carried out covering a width of about 20 m and a length of 150 m outside of the mine and 10 m inside of the mine area.

The first sample collection was performed inside the mine area at a distance of around 10 m away from the mine boundaries. The second sampling position was again inside the mine, 1 m away from the mine boundaries. The last four sampling positions were outside of the mine at a distance from the mine boundaries of 1 m, 10 m, 15 m, and 150 m respectively. So from each position and along the three transects, *A. tenuis* and *F. rubra* populations were selected.

Populations of the same plants were also taken for the purpose of comparison from an uncontaminated area, 5 km away. These were used as control populations.

Since Bradshaw (1952) had reported that a period of growth in normal soil does not reduce heavy metal tolerance, the plants were propagated in plastic beakers containing JOHN INNES soil. These materials were kept in a green-house for about 8-10 weeks until their development was fulfilled and they became ready for checking. The temperature of the green-house was almost steady ranging between 25-30°C, whereas the photoperiod was about 16 hours per day and reinforced by artificial light during the winter or on dark days.

TEST FOR HEAVY METAL TOLERANCE

It is well known that the toxic action of heavy metals against plants can be expressed in several ways. The method used for the measurement of tolerance was limited to the test of root production, because it is the quickest, most characteristic and convenient way of measuring the effect of metal toxicity (Gregory and Bradshaw 1965, McNeilly and Bradshaw 1968, Bradshaw 1970). It has been thus found that the toxic action of heavy metals is manifested as inhibition to root development when in relatively low concentrations, but as a complete stoppage of growth at higher concentrations.

Healthy tillers from a single tuft of each plant population, growing for 8-10 weeks on normal soil in a green-house, were used for testing. The roots were removed from tillers leaving only a basal node capable of root production.

The technique was based on the works of Bradshaw (1952), Wilkins (1957, 1960), Jowett (1958, 1964) as modified by McNeilly and Bradshaw

(1968). A detailed description of this method was given in previous papers (Karataglis 1976, 1978a, b 1980, 1982).

The tolerance index was expressed by the ratio of the mean length of the longest roots in different concentrations of toxic solutions to the mean length of the longest roots in the nutrient solution. The following ratio pictures the index of tolerance (I.T.).

$$\text{I.T.} = \frac{\text{mean length of longest root in solution with metal}}{\text{mean length of longest root in solution without metal}} \times 100$$

SOIL ANALYSIS

The soil to be analysed was collected from the rhizosphere of plant samples, at a depth of 6-12 cm from the soil surface. All soil sampling was treated in a similar way as that formerly described in detailes (Karataglis 1981, 1982).

The pH values of the mine samples ranged between 6.8-7.3. The amounts of heavy metals were measured by atomic absorption. Three measurements were made and the levels of metal contaminants were:

Mine populations	Control population
Zinc 3.500-22.000 ppm	50-90 ppm
Lead 2.000-5.000 ppm	40-110 ppm
Copper 50-110 ppm	40-80 ppm

RESULTS

Checking the tolerance of populations collected from inside the mine is pictured by Figures 1, 2, 3 and 4. These indicate that populations of both species (*Agrostis* and *Festuca*) can tolerate Zn as much as Pb. These elements (Zn and Pb) also exist in the soil thus accounting for the high index of tolerance (Karataglis 1978a, b, 1981, 1982). In contrast, copper concentration in the soil is at normal levels, while the index of tolerance towards Cu, of the Zn and Pb tolerant populations mentioned above, does not differ from the index of tolerance of the control population.

We further checked the tolerance of populations within the mine from the first sampling position (10 m away from the boundary) and from the second one (1 m away from the boundary) and came up with high tolerance indices. Any differences observed between the tolerance indices of these two positions lie within our expectations as far as ranging is concerned. In addition, all sampling from the first position outside the mine differed little from the tolerance indices yielded by the last two collections from

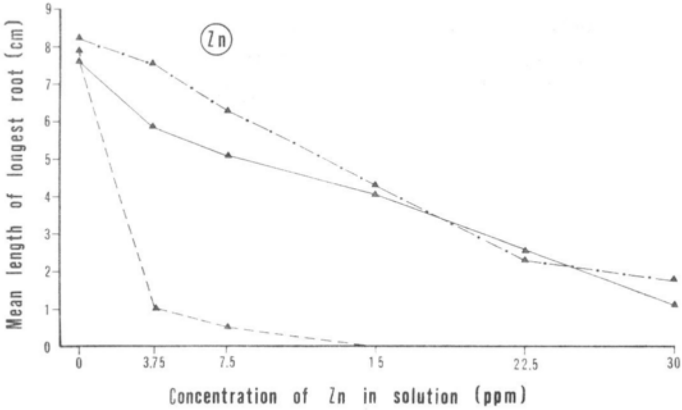

Fig. 1. The effect of increasing concentrations of Zn on the root length of *Agrostis tenuis* populations, collected from mine (1), (2) and uncontaminated areas (3)

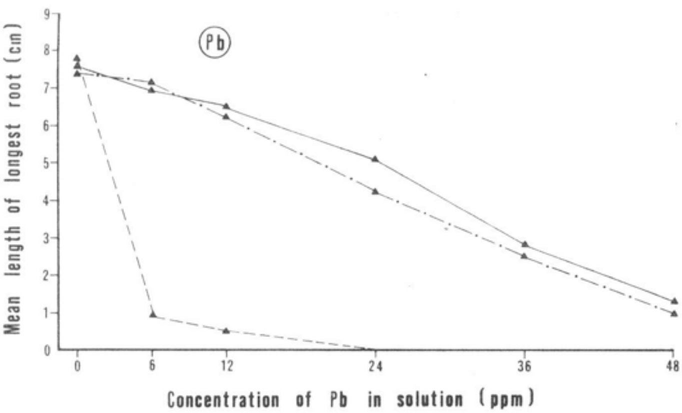

Fig. 2. The effect of increasing concentrations of Pb on the root length of *Agrostis tenuis* populations, collected from mine (1), (2) and uncontaminated areas (3)

inside the mine. In the ensuing collections, tolerance appeared to be characteristically lower (see Figs. 5, 6). Populations grown at a distance of 150 meters away from the mine boundaries demonstrated relatively high tolerance despite the fact that the Zn and Pb concentrations in the soil were quite low.

Plant behaviour towards Cu did not present any changes both inside and outside the mine, since the copper concentration of the soil was insigificant in all cases.

Field observations concerning the period of plant flowering inside and outside the mine turned out to be quite interesting. In relatively remote populations that might be considered to be pasture, one can trace individuals which keep producing flowers when the tolerant plants of mines have already ceased to flower. One-third to one-fourth of the tolerant populations bloom sooner than the pasture ones. This ratio decreases if we compare

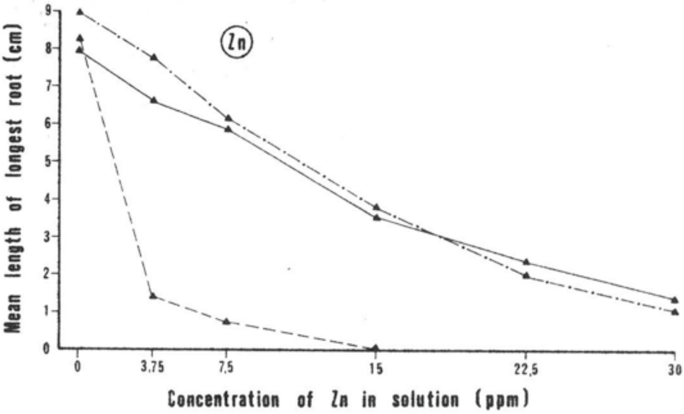

Fig. 3. The effect of increasing concentrations of Zn on the root length of *Festuca rubra* populations. collected from mine (1). (2) and uncontaminated areas (3)

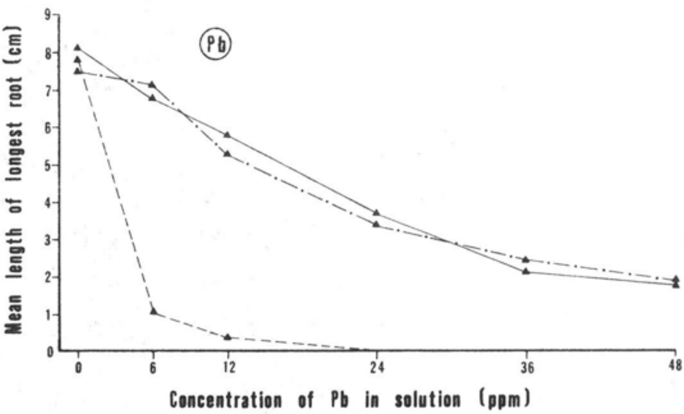

Fig. 4. The effect of increasing concentrations of Pb on the root length of *Festuca rubra* populations. collected from mine (1). (2) and uncontaminated areas (3)

tolerant populations from a mine with others found at the mine boundaries, which means that the time difference in flowering becomes shorter. Things become even more complicated if we want to determine the time difference in the flowering of successive sampling positions on a transect line.

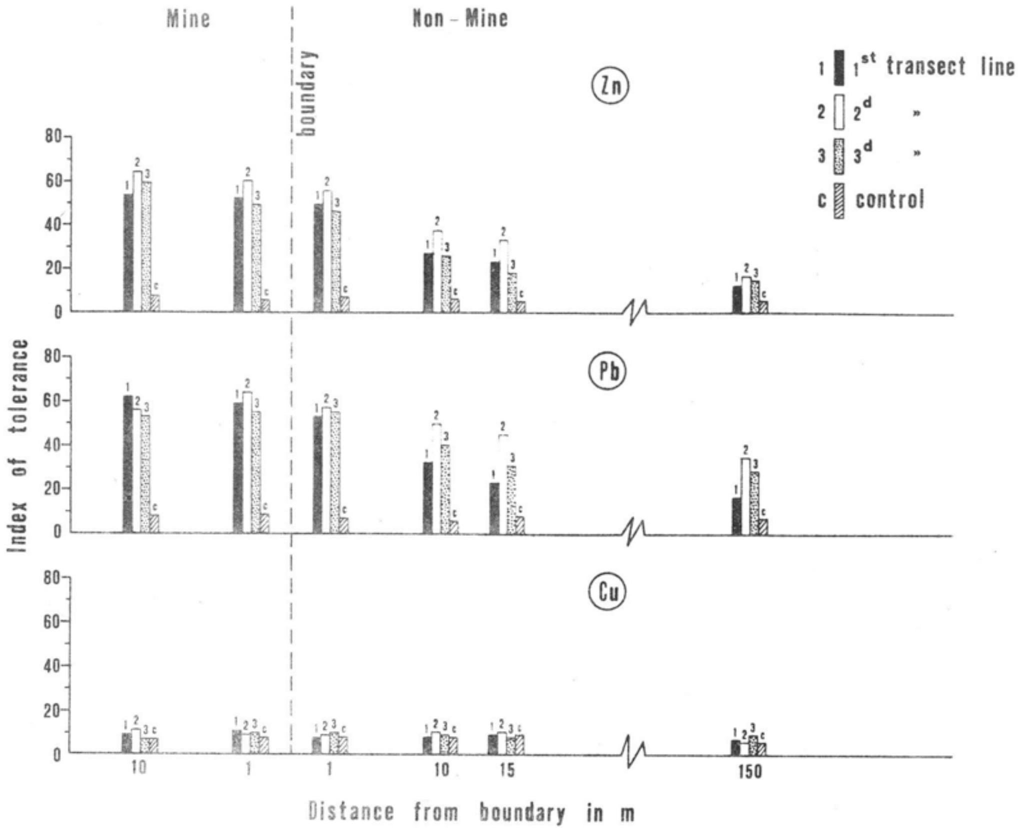

Fig. 5. Mean index of tolerance of mine and non-mine populations of *Agrostis tenuis* across three transect lines

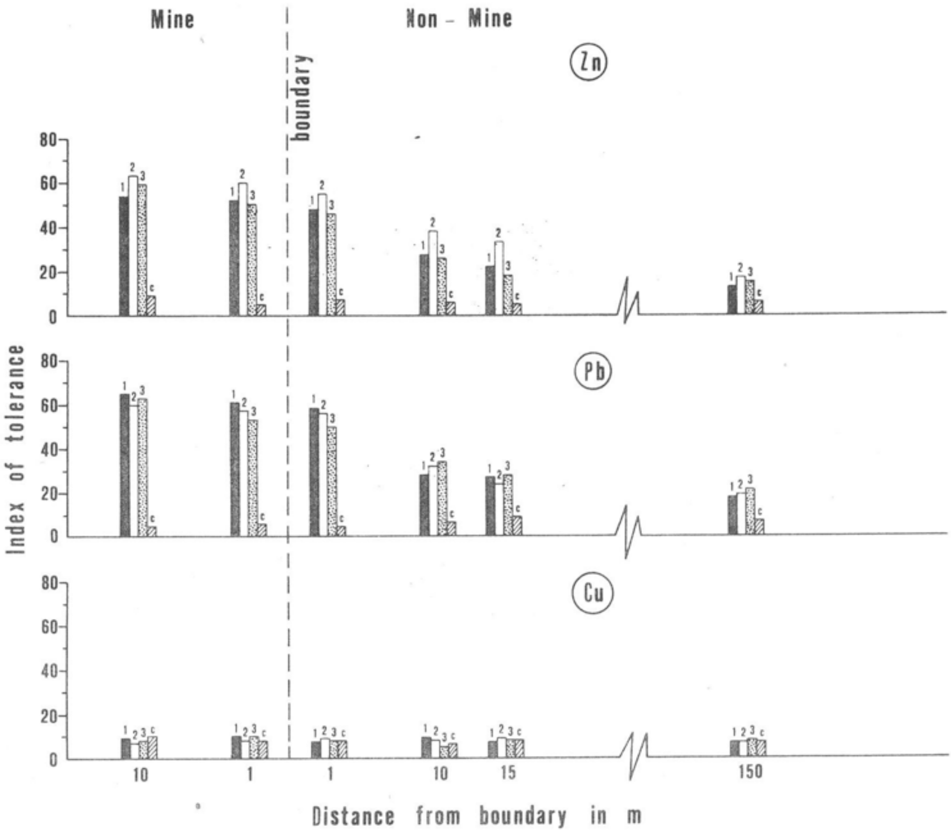

Fig. 6. Mean index of tolerance of mine and non-mine populations of *Festuca rubra* across three transect lines

DISCUSSION

Gene flow can be appreciated well if we examine the difference in the degree of tolerance between natural progeny and parents (Karataglis 1980). It is even easier for someone to estimate the extent and degree of gene flow between two parapatric populations, if one studies the tolerance of adult individuals in combination with the presence of any toxic metal in the soil.

Theoretically one should expect a characteristically abrupt decline of the tolerance index of the populations found very near but beyond the mine boundaries. At a very short distance, the tolerance of these populations should no longer exist, provided that there is no toxic metal in the soil. Nonetheless, things are different in everyday practice and there are cases where neighbouring mine populations show tolerance even if the soil contains no toxic metal (McNeilly 1968, Khan 1969, Karataglis 1980).

This fact was attributed to the presence of gene flow, that is, to the transport of the genetic material of the plants already selected from the mine to the surrounding non-tolerant populations. It is obvious that if during the transport no natural or artificial obstacles interfere, then after some years the character of tolerance will move along away from the mine, which is of great meaning for those cases in which future populations will have to evolve in rising pollution or any other environmental stress (Bradshaw and McNeilly 1981).

It is thus concluded that if there are one or more toxic metals on a mine site, then it is possible to select individuals tolerant to the corresponding toxic metals (Walley et al. 1974, McNeilly 1979, Karataglis 1982). According to our experimentation, the plants grown inside the mine showed tolerance only to Zn and Pb. On the basis of such a result, we are to assume that these plants evolved through selection to existing environmental conditions. None of the individuals examined from the different populations showed tolerance to Cu, since this metal was completely absent from the soil. Similar behaviour was displayed by the populations of the closely neighbouring environment. In fact, the investigation of the plant tolerance yielded a gradual decrease combined with the distance in sampling. The behaviour of the populations inside the mine as well as of the ones outside the mine towards copper was steady and similar to that of the control (Figs. 5, 6).

Transport of the genetic material by gene flow does not follow a one-way direction. It is true that genetic material can be transferred from pasture to mine populations, in which case the produced individuals, if having a decreased tolerance will neither develop or develop very little and then will be destroyed (Walley et al. 1974) since the selective factor termed "toxic soil" is dominant on this site. The occurrence of some individuals on the mine boundaries with a low index of tolerance is explained in this way as well as the high degree of tolerance of the individuals from the first collection outside the mine in combination with the most distant areas. In Figs. 5, 6 the index of tolerance decreases as the distance from the boundary increases, due to the reduction of gene flow (Gleaves 1973, Karataglis 1980). The high levels of tolerance expressed by non--mine populations, even if this soil was found to be poor in heavy metals, suggest gene flow from the mine.

Any differences observed during the period of flowering between populations inside and outside the mine may be due to their being adjusted to local ecological conditions, or to their having been grown there as a result of gene flow specifically known as an isolating mechanism. Irrelevant to whether the cause is the aforesaid hypothesis or any other supposition, it is a fact that selection has furnished differences in the time of flowering, resulting in the occurrence of isolating mechanisms developed not only by distant but by neighbouring populations as well thus slowing down gene flow. Consequently one of the simplest mechanisms contributing to the gene flow reduction between populations is a difference in the time of flowering.

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Przepływ genów w populacjach parapatrycznych Agrostis tenuis L. i Festuca rubra L.

Streszczenie

Badano przepływ genów między populaciami *Agrostis tenuis* L. i *Festuca rubra* L. na obszarze kopalni cynku i ołowiu, i na terenach sąsiednich, w odległości 150 m od granic kopalni. Okazało się, że populacie rosnące na terenie kopalni wykazuią wysoki indeks tolerancii na Zn i Pb, na pierwiastki które znaiduią się tam w glebie. Populacie rosnące poza obszarem kopalni wykazuią stosunkowo wysokie indeksy tolerancii na te pierwiastki, pomimo że nie ma ich w glebie. Z drugiej strony, indeks tolerancii na miedź (nieobecną w glebie) populacii na terenie kopalni i na obszarach przyległych był bardzo niski i nie różnił się od wartości kontrolnych. Różny czas kwitnięcia tych populacii parapatrycznych utrudnia przepływ genów, powodując izolację populacii sąsiadujących ze sobą.