Climatology and genetics – is there any interface? An example of forest trees

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Abstract

In addition to the role of Gregor Mendel as a biologist and founder of genetics, he also devoted a part of this scientific life to weather observation and climatology. This study focuses on possible meeting points of these two roles. On the example of forest trees, it shows how climate information can be useful for population and evolutionary genetics and vice versa. Four studies are used to illustrate this relationship: Holocene migration of beech, genetic variation in Serbian spruce, assessment of adaptive variation in beech in a common-garden experiment, and epigenetic phenomena in Norway spruce.

Key words: local adaptation, postglacial migration, genetic drift, forest trees

Introduction

When the first edition of Charles Darwin's *Origin of Species* (Darwin 1859) appeared in November 1859, it caused a true revolution in science as well as in society. It was not because Darwin 'invented' evolution (as often suggested by laymen); actually, the idea of biological evolution has been present in naturalist thought long before, starting from Anaximandros, Herakleitos and Empedokles over Buffon to Lamarck (Larson 2006). Darwin's main contribution to biology was the population view of biological change: instead of looking on trait expression in a particular pair of parents and the set of their descendants, Darwin focused on variation in species and populations as large sets of individuals, integrating the available knowledge in geology, biogeography and descriptive biology (Dawkins 2009). Consequently, he was the first to suggest a

mechanism underlying evolution, which was able to withstand not only ideological opposition, but also scientific proof, although many aspects and details of his ideas needed to be corrected later. Maybe this was the reason why darwinism provoked vivid controversies lasting until today.

Gregor Mendel owned an outprint of Darwin's book and hand-written remarks on margins document that he studied it with attention and interest (Fairbanks and Rytting 2001). The opinions about Mendel's attitude towards the evolutionary theory diverge, reaching from displaying Mendel as a categorical opponent of darwinism up to portraying him as a good Darwinian (Sapp 1990 and the citations therein). Therefore, today we can only speculate about his motivation for the experiments with plant hybridization, which laid the foundations of genetics (Mendel 1866). He might have intended to provide evidence for (Fisher 1936) as well as against evolution (Bishop 1996). Whatever is true, the fact that hereditary information is transmitted from parents to offspring in discrete units without alteration by the environment has long been used as an argument against evolution. Only the development of quantitative and population genetics in the 1920s and the 'new evolutionary synthesis' in the 1930s–1940s showed that not only there is no contradiction between genetics and Darwinism, but Mendelian inheritance is a basic prerequisite for evolution by natural selection (Fisher 1930, Wright 1931).

Mendel is thus known to everybody who passed secondary school anywhere in the world as the founding father of genetics. This conference is, however, devoted to Mendel as a meteorologist and climatologist (a less known role, but clearly documenting his prominence in natural sciences). So, is there any link between the study of climate and the study of heredity?

There is. Returning to the first paragraph: the keyword is 'evolution'. Mendel himself did not integrate the mentioned two parts of his scientific career, but his successors did. The fact that climate is the main driver of natural selection and largely determines not only biogeographical patterns, but also distribution of genetic lineages and composition of gene pools within a species (especially in plants), has been generally recognized since the foundation of population genetics. On one hand, various components of climate act as factors of

selection and provoke local adaptation; this process has broad practical implications in the light of the ongoing climate change. On the other hand, as climate sets limits for persistence of a species at a particular location, it determines population sizes, migration barriers and corridors, stepping stones for gene flow and other factors driving neutral processes in populations and thus shaping their gene pools. Some components of climate even directly affect the hereditary material: polyploidy is correlated with temperature regime reflected in latitudinal or altitudinal gradients (Ramsey and Ramsey 2014), ultraviolet radiation causes non-homologous recombination in plants (Ries et al. 2000) etc.

Forest trees represent an exemplary case of organisms where the interactions between genes and climate are of direct practical relevance. In contrast to agricultural plants, forest trees are practically undomesticated. Moreover, natural stability is expected from forest ecosystems, including commercial forests managed by man. Trees are long-lived organisms, which have to cope with environmental fluctuations, pathogen and pest occurrence and other stresses during their long life span. A sufficient genetic variation of tree populations, sufficient adaptedness to present environments and adaptability of their gene pools to future changes are basic prerequisites of resistance and resilience of forest ecosystems. Therefore, knowledge of genetic variation patterns and their historical as well as current relationships to climate are of essential importance for forestry.

This contribution shortly summarizes four studies documenting the relationship between gene pool composition and climate. First two studies show how climate change in the past shaped ranges and genetic variation levels of tree species and how genetic tools can be applied to reconstruct these changes. Latter two studies document how climatic adaptation is reflected in fitness-related phenotypic traits and what may be the hereditary basis of this adaptation.

Materials and methods

The first study focuses on the colonization of the range of European beech (*Fagus sylvatica* L.) during the Holocene (Magri et al. 2006). It is based on the analysis of 608 populations represented by >50 trees each and genotyped at 7

allozyme loci. Spatial analysis of variance (SAMOVA) based on a simulated annealing procedure was applied to the genotype set to define groups of populations that are geographically homogeneous and maximally differentiated from each other. The outcomes were combined with the reconstruction of postglacial range expansion based on fossil pollen and macrofossils.

The second study focuses on genetic consequences of range fragmentation caused by the Holocene warming in a Balkan endemic, Serbian spruce (*Picea omorika* Purk.). Levels of genetic variation were assayed in 13 populations genotyped at 16 allozyme loci (Ballian et al. 2006) and a mitochondrial marker (*nad1-2*).

In a study of adaptation we examined reactions of 87 beech populations on geographical and climatic transfer in a common-garden experiment. Populations of different origin (provenances) were planted in 24 trial plots over Europe in 1998. A coordinated assessment of growth and survival was accomplished in 2007. Height growth and survival rates of individual provenances were fitted against transfer rate (difference of geographical coordinates or climatic variables between the site of plantation and the site of origin) to estimate optimum site and optimum transfer rate, where fitness attains maximum.

Finally, the effects of climate of the site of juvenile growth on later behaviour of conifer provenances were assessed in a nursery trial. Twelve provenances of Norway spruce (*Picea abies* Karst.) were sown in two climatically contrasting nurseries located at altitudes of 350 and 1100 m a.s.l., respectively. After the first year, half of the material was reciprocally transferred between nurseries, and budburst phenology was assessed at the beginning of third year.

Results

The study of genetic differentiation of beech in Europe revealed 9 lineages (SAMOVA groups), which are, however, not equally differentiated. A comparison of the distribution of lineages with paleobotanical evidence (dating of fossil pollen >2% of the pollen spectrum or macrofossils on ^{14}C-calibrated sites) revealed that they correspond to glacial refugia or refugial areas, which contributed to the recolonization of the continent enabled by climate improvement during the Holocene. The major part of the range was colonized

from a single source population located at the eastern foothills of the Alps and in Istria (fig. 1; red lineage).
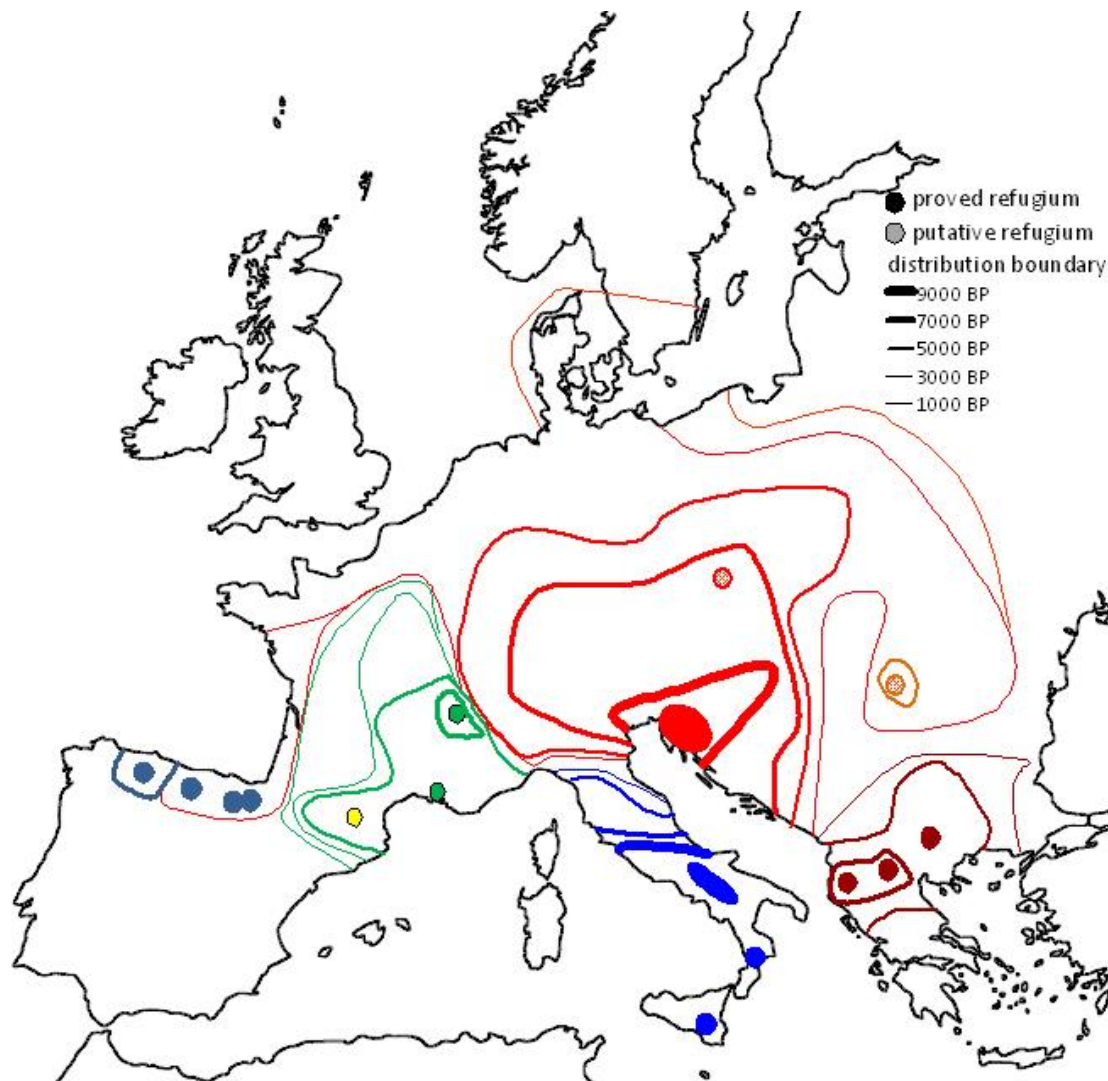

Fig. 1 Schematic drawing of the distribution of genetic lineages of common beech and their expansion during the Holocene

This refugial population spread into all directions and succeeded to colonize northern Europe, most of the Alps, the Carpathians, the Atlantic coast, and even succeeded to invade the Pyrenees and overlay the local refugia. Another major refugial population survived the last glacial in southern Apennines, Calabria and Sicily (blue lineage). This population started to expand very early, but colonized only the Apennine peninsula. Several refugia were located in the Maritime Alps, Massif Central and eastern Pyrenees. Although differentiated, all they belong to

the same clade and colonized the western Mediterranean area (green/yellow group). Finally, southern Balkans harbours three lineages also belonging to the same clade and representing probably several refugial populations (brown group). Two lineages represented in the Cantabrian range and the Southern Carpathians (Apuseni Mts.) are indicative of local secondary refugia.

The case study of *Picea omorica* was used to demonstrate the effect of range fragmentation. In spite of being a stenoendemite, Serbian spruce is highly polymorphic at the mitochondrial *nad1* gene, 7 haplotypes were found. Most populations were monotypic in the 295 bp allele. However, the largest population on Veliki Stolac lacks this haplotype completely. Similar contrasts were observed for frequencies of allozyme alleles; e.g. the allele *Mdh-B/100* represented in the population Goštilja by 87% is completely lacking 12 km apart in Tovarnica (Ballian et al. 2006). The coefficient of differentiation F_{ST} of 0.261 only illustrates a high overall differentiation level (commonly $F_{ST} \approx 0.05$ in widespread conifers). On the other hand, within-population variation is low, as shows the comparison to a Norway spruce population from the Poľana Mts. (Table 1). Even when all *P. omorica* populations are pooled together, allelic richness ($A_{[20]}$ and $A_{[400]}$; number of alleles recalculated for common sample size of 20 and 400 gene copies, respectively, by rarefaction) is only a half compared to *P. abies.* The same applies to proportion of polymorphic loci (*PP*) and gene diversity measured by expected heterozygosity (H_E).

Table 1 Genetic variation in *Picea omorica* compared to *P. abies*

Species	N	$A_{[20]}$	PP	H_E
P. omorica				
average	33.7	19.11	20.9	0.067
pooled	400	22.24*	43.8	0.088
P. abies	200	42.00*	81.3	0.140

*Rarefaction to *g*=400 gene copies

Neutral processes such as genetic drift or gene flow affect also adaptive variation. Although the recent progress in functional genomics allowed identification of genes underlying phenotypic traits and polymorphisms within these genes causing allelic variation, mapping of adaptive genetic variation is still at the very beginning and for practical purposes, we have to rely on the assessment of the genetic component of fitness-related trait variation in common gardens such as provenance experiments. In the case of the international beech experiment we assessed how provenances respond to transfer by height growth and survival. An illustration of the geographical distribution of optimum transfer rates of beech provenances as measured by their height-growth and survival responses is shown in Fig. 2.
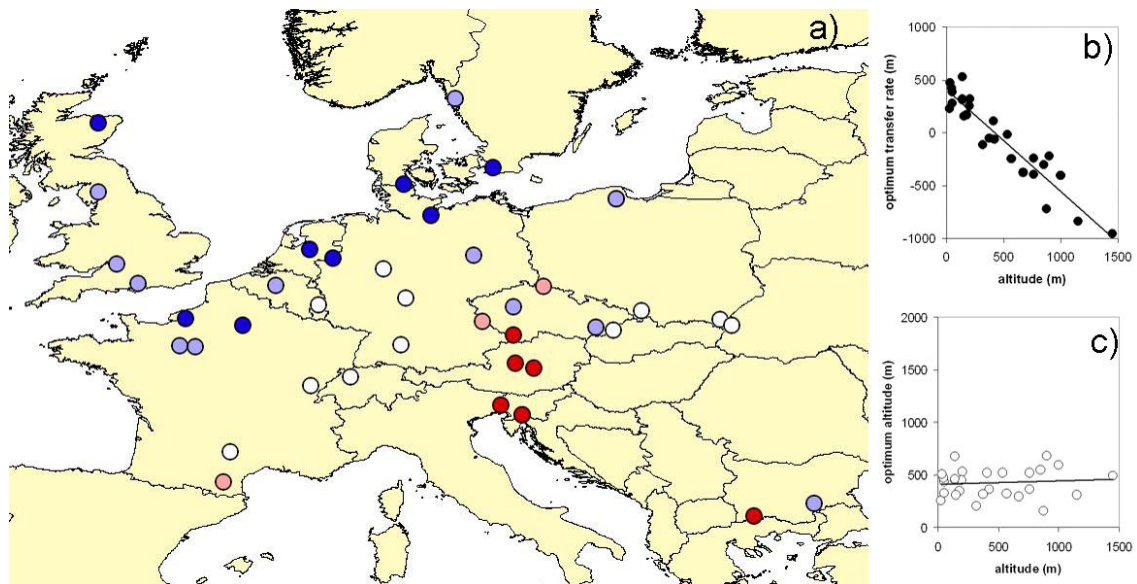

Fig. 2 Height growth responses of beech provenances to altitudinal transfer: a) optimum transfer rates (blue and red dots: provenances preferring transfer to higher and lower elevations, respectively), b) regression of optimum transfer rates on altitude of origin, c) regression of optimum altitude on altitude of origin

The pattern is spatially continuous, i.e. spatially proximate populations mostly behave in a similar way: those from mountainous region (Alps and Carpathians) show different preferences compared to lowland populations distributed along

the Atlantic coast. Naturally, there are outliers of these general trends, mainly provenances represented in few trial plots (i.e., for which the estimation of regression parameters is less reliable), extremely marginal or non-indigenous. The correlations between transfer rates and the underlying ecological variables are mostly highly significant and negative, meaning that transfer towards the centre (in terms of geography) or towards moderate conditions (in terms of climate) is generally preferred by beech (Table 2). On the other hand, correlations for the optimum site are generally non-significant.

Table 2 Correlations between optimum transfer rates/optimal climates and underlying environmental variables for provenances

Climatic/ geographic variable	Height		Survival	
	Optimum transfer rate	Optimum position	Optimum transfer rate	Optimum position
Longitude	–0.368 ns	0.316 ns	–0.329*	0.165 ns
Latitude	–0.925***	0.326 ns	–0.374*	0.246 ns
Altitude	–0.668***	0.057 ns	–0.850***	0.082 ns
MAT	–0.590*	0.573*	–0.675***	–0.116 ns
Precipitations	–0.762***	0.289 ns	–0.544***	0.166 ns
EQ	–0.684***	–0.100 ns	–0.108 ns	0.031 ns

Although expression of a gene in a phenotypic trait may depend from the environmental context, the hereditary material as such should not be affected by the environment. To verify whether it is so we organized a transplant experiment where samples from a population with identical gene pools were initially exposed to different climates (warm/cold) and later their budburst dates were compared. Expectedly, Norway spruce provenances scored in the cold nursery flushed later than those in the warm nursery. However, budburst date was also strongly influenced by the climate of the nursery, in which the material spent the first year of life. Independent of scoring site, all provenances germinating in a warm nursery flushed on average 2 to 7 days later than those growing the first vegetation season in warm climate (Fig. 3)).
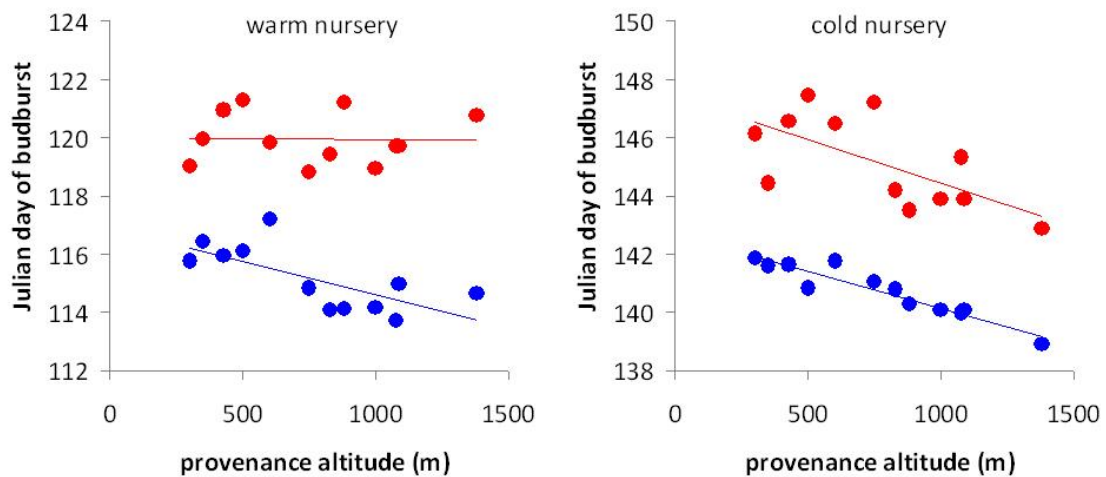
Fig. 3 Regressions between budburst date and altitude of origin for seedlings grown the first year in a warm (red) and cold (blue) nursery, scored after transplantation in the warm (left) and the cold (right) nursery

Discussion

The history of populations reflecting population sizes, migration paths and gene flow directions and levels largely determines their present gene pool. Holocene climate changes allowed expansion of tree populations from refugia, where temperate tree species were confined during the glacials, but not all refugial populations were able to profit from this opportunity. The study of European beech points to three facts. First, in contrast to previous paradigm of paleobotany, it demonstrated that effective refugia of temperate species in Europe could have been located farther to the north than on the three main southern peninsulas. Second, it showed that large mountain chains such as the Alps or the Carpathians need not act as migration barriers, as generally postulated (e.g., Taberlet et al. 1998); in the case of beech, it was rather main European rivers and their surrounding lowlands (the Po valley, the Hungarian and Wallachian plains along the Danube) which prevented expansion of the Apennine and Balkan lineages. Finally, it showed that refugial populations in the Cantabrians and Southern Carpathians were unable to expand, although climate improved also here, and were overlaid by newcomers from the main Slovenian refugium. The probable cause was a small population size, leading to

gene pools depleted of adaptive alleles and high inbreeding levels, both factors decreasing fitness.

Genetic continuity in widespread tree species such as beech is in a sharp contrast with the endemics with fragmented range. Chaotic patterns of genetic differentiation and low diversity are signs of genetic drift in almost completely isolated small demes. On the other hand, the presence of several genetic lineages in an endemic indicates a larger range in the past. Moreover, Serbian spruce is a species untypical for its present range – the growth habit (tall trees with short branches and slender crowns) indicates that the species developed in areas with a high snow cover. At present it grows in gorges and north-oriented slopes that receive almost no direct sunlight; the microclimate of these sites is characterized by very high air humidity, high precipitation regularly distributed over the year, high snow cover, and low winter temperatures (Vidaković 1991). Refugial areas in the Bosnian and Serbian mountains influenced by the Mediterranean climate with hot and dry summers may thus have become a trap. With the onset of the Holocene warming, the spread of Serbian spruce into climatically more friendly areas could have been hampered by a lack of migration corridors or stepping stones in the close vicinity of the refugia.

Two criteria have been proposed as diagnostic of local adaptation in common garden experiments: local deme should be superior to foreign demes ("local vs. foreign"), and a deme should show higher fitness in its own habitat compared to other habitats ("home vs. away") (Kawecki and Ebert 2004). Provenance experiments allow assessing the response of tree populations to environmental changes simulated by the transfer to different ecological conditions. Response measured by any fitness-related trait is commonly non-linear; transfer exceeding the optimum rate commonly results in lower growth or survival. Under local adaptation, the optimum climate is identical (or positively correlated) with the climate of origin, meaning that optimum transfer rates are expected to be close to zero for each provenance. In contrast, if the optimum climate is the same for all provenances, optimum transfer rates are expected to be negatively correlated with the climate of origin. The presented variation patterns of fitness components (juvenile survival) or fitness-related traits (height growth) in a large-

scale provenance experiment with common beech do not give clear indications for the local adaptation, considering the "home vs. away" criterion *sensu* Kawecki and Ebert (2004). The lack of local adaptation may be caused by considerable phenotypic plasticity of beech, allowing it to avoid selection pressures exerted by climate. Alternatively, the time (number of generations) elapsed since the colonization of most of the present range during the Holocene may have been insufficient to develop adaptive differentiation under extensive gene flow.

There are, however, traits always giving indication for local adaptation in practically all provenance experiments, namely those associated with vegetative phenology. Timing of budburst, budset, leaf discoloration etc. generally shows smooth change along geographic and climatic gradients, which has typically been attributed to adaptation by natural selection (Wright 1976). However, both the empirical experience with transferring seed orchards southwards and experimental studies conducted during the last 20 years have demonstrated that climatic conditions during sexual reproduction affect vegetative phenology and frost hardiness (and consequently growth) in conifers (Johnsen et al. 2005): progenies possess a memory of temperature and photoperiod during the embryonal development and seed maturation, whereas the climate during prezygotic stages, i.e. micro- and macrosporogenesis and fertilization itself, does not affect phenological behaviour of the progenies. It has been shown that such memory effects very probably rely on changes of gene expression Yakovlev et al. 2010), although the underlying molecular mechanisms have not been clarified yet. The presented outcomes of the nursery experiment show that the modifying effect of environment need not be restricted to embryonal development, and carryover effects can be provoked by the climate during juvenile growth as well: heredity going beyond Mendel's rules.

Conclusion

Mendel himself surely did not anticipate how big branch of science and business will once grow out of his discoveries. His crossing experiments

focused on phenotypic traits (logically), but were indispensable for later explanation of cytological and molecular mechanisms of heredity. As mentioned in the Introduction, Mendelian genetics is regarded as indispensable for explaining, how plant population adapt to changes of climate, even without knowing exact details about the molecular basis of this process. This is, however, not all: molecular tools, currently widely used in ecology, biogeography and other fields to follow the interactions between biota and climate *sensu latissimo*, all have their roots in Mendel's experiments. Even if he had never made a single air-temperature measurement, Mendel's contribution to understanding the climate and its effects on living world would be undisputable.

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Acknowledgement

Summary

Gregor Mendel je obecně znám především jako zakladatel genetiky, jeho práce v meteorologii a klimatologii vešla do obecného povědomí v podstatně menší míře. Tento příspěvek je věnován hledání styčných bodů obou těchto aspektů Mendelovy vědecké dráhy. Na příkladu lesních dřevin ukazuje, nakolik jsou informace o klimatu a jeho změnách nutné pro populační a evoluční genetiku, a naopak, nakolik mohou genetické nástroje dopomoci pochopení vývoje klimatu i jeho dopadů na živá společenství. Pro ilustraci jsou použity studie postglaciální rekolonizace areálu buku lesního v Evropě, genetických dopadů klimaticky podmíněné fragmentace areálu u smrku Pančićova, hodnocení adaptivní variability buku lesního v přesazovacím pokusu a epigenetická variabilita vegetativní fenologie u smrku ztepilého.

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