

Application for funding – INTERACT Transnational Access

1. Basic data

Project name: Aspen genetic patterns

Project acronym: AGeP

Name and contact information of group leader

Dr. Berthold Heinze; Federal Research Centre for Forests (BFW), Austria:

Department of Genetics, Hauptstrasse 7, A-1140 Vienna

berthold.heinze@bfw.gv.at – tel. +43 1 87838 2219

Site of research: Mukhrino Field Station (Russia)

Duration of project (person-days, dates):

Effort: 20 person-days (2 persons, 10 days each)

Timing: 10 days in late July / early August 2013; precise dates to be agreed with station

Date of the specific INTERACT call:

Boreal Stations for summer 2013 and winter 2013/2014; call open 1-28 February 2013

2. Background

Forest trees were quick to re-colonize areas that became accessible to them after the end of the last glaciations. This is particularly true for ‘pioneer’ species like the Eurasian aspen (*Populus tremula* L.), which in our days inhabits an immense range from the very western to the very eastern end of the Eurasian land mass (see map in e.g. Dickman and Kuzovkina 2008). The refugial areas during the glaciations, as well as presumable routes of re-colonization, can often be deduced from fossil pollen finds; this is, however, not easily possible for members of the genus *Populus* because the pollen is too fragile. Moreover, the eastern distribution of *P. tremula* is often considered as a separate species, *P. davidiana* (e.g. von Wuehlisch 2009). While finding out about re-colonization routes and times used to be a somewhat academic exercise for some time, the recent global focus on climate change adds a dimension of forecasting to this: more precise knowledge on how, when, from where, and why trees were able to re-colonize such vast land masses may help in understanding how current and future range expansions due to global warming may proceed. Genetic data, produced from markers in e.g. chloroplast DNA, can help in elucidating past events in re-colonization (e.g. Fussi et al. 2010).

I have over the past few years started to collect and analyse biological samples of this species which were sent to me by a number of helpful colleagues, or collected by myself during previous visits to Siberia. The general picture that emerged from these experiments is that *P. tremula* may have colonized the larger, western part of this range from residual, confined stands in Europe; while a separate, eastern re-colonization route can be assumed for extant populations east of Lake Baikal (Heinze 2011). During this expansion of its range, the diversity of chloroplast genetic types (so-called haplotypes) apparently decreased by essentially stochastic processes. We have also studied clonal populations of this and related species, and have found that relatively large clonal stands (produced by root suckers) are often the response to specific environmental (harsh) conditions (e.g. Heinze and Fussi 2008, Van Loo et al. 2008, Fussi et al. 2012). The proposed research will give an opportunity to reconcile patterns of large-scale and small-scale range expansion by studying populations of

aspen close to or at the range limit, where harsh growth conditions may lead to increased clonality and metapopulation dynamics, and at scales that range from individual stands to large river floodplains. This will add significantly to recent findings on the re-colonization of Scandinavia by this species (De Carvalho et al. 2010), where the hybridization of different invading lines may have created specific patterns of environmental adaptation.

3. Objectives

The hypothesis of the planned research work is that populations and stands in the vicinity of the field station and further north will still carry a genetic signal of the re-colonization process in that their chloroplast haplotype (genetic) patterns will fit into the previously established general picture - decreasing haplotypic richness from West to East and from South to North, and that metapopulation dynamics will be present at the range limit and include a role for clonal stands. It is also expected that they harbour decreasing levels of genetic diversity (intra-species biodiversity) with increasing environmental hardship caused by less suitable growing conditions, changes in photoperiodic and temperature regime, biotic pressures and water saturation, etc.

The objective of the proposed field trip is to collect material (suitable for extraction of DNA, like leaves, buds, or cambium) from trees in a number of stands around the field station, and further along northwards to the edge of the species' distribution, and at the same time to assess the main environmental parameters of the specific sites – altitude, site characteristics related to water (water table, seasonal flood levels, distance from river arms), soil and vegetation type, tree density, tree ages and sizes, tree health status, recruitment of saplings, etc. The genetic diversity parameters (chloroplast DNA haplotypes and diversity in nuclear genes) will be determined after the visit in the applicant's laboratory (funded by sources other than INTERACT) and will follow established procedures (e.g. Fussi et al. 2010, De Carvalho et al. 2010, Ma et al. 2010). The hypothesis will be tested by comparing characteristics of tree populations and their sites with genetic patterns, in a statistical design.

The proposed work will shed further light on the drivers of macro- and micro-distribution patterns in one of the most widespread forest tree species in Northern Eurasia, thus tying field work at the station to larger investigations in ecology and biodiversity on a continental scale (by integrating the work with previous and ongoing investigations in this species). All trees and sites will be georeferenced, and thus accessible for further (e.g. ecological) investigations.

4. Research methods and material, ethical issues, permits

Work at the field station will mostly consist of sampling biological material from aspen trees. I will rely on the station staff to guide me to stands of such trees. These stands will be characterized by measuring (height, girth), describing (habitus, health status; if possible, recording sex) and counting the trees, describing the prevalent vegetation type, and gathering information on specific characteristics (soil, water regime, temperature conditions). Fresh leaves, or cambium, or buds, whatever is available, will be collected from trees separately, and will immediately be dried in silica gel. A few leaves, buds, or cambium 'punctures' (of approx. 1 cm diameter, obtained with a hollow punch) will suffice per tree. GPS coordinates of single trees or stands will be recorded with a hand-held device. We will try to identify a number of stands in the vicinity of the research station, and further stands north towards its northern limits. The aim is to collect up to approximately 200 or 250 specimen (trees).

After the field visit and back in the Austrian lab of the applicant, these samples will be subject to DNA extraction, and analysis of genetic markers following mostly Fussi et al. (2010), Heinze (2011), Fussi et al. (2012) and Ma et al. (2010). Chloroplast haplotypes will tell whether the previously observed patterns of less variation towards the East can be upheld. For individual stands, tests of clonality with so-called microsatellite DNA fingerprints will test the hypothesis of more clonality in harsher growing conditions.

The proposed research does not raise any ethical issues.

Permits for exportation of the collected plant material will be obtained in due course before the visit (with the support of the research station staff).

We do not anticipate any problems with the collection of the material or the data.

Additionally interesting INTERACT stations where similar collections could be made are Numto Park (also accessible from Khanty-Mansiysk), and Spasskaya Pad Research Station near Yakutsk in Siberia, but these are not participating in the current call.

5. Implementation

The estimated budget as below is based on two persons (B. Heinze and a young researcher from my group; either Daniela Jahn, age 29, or Manuel Gomez Mazuecos, age 26 years) travelling to the field site, spending about four days with sampling and measurements, and then travelling north with field station staff for another four or five days in order to investigate further stands of the species. Timing is flexible (as several types of biological material can be collected alternatively); the field station manager indicated that late July and early August may work best.

Item	per person	total
air travel	Vienna-Moscow-Khanty-Mansiysk and back: c. 1200 € (depending on exact dates)	2400 €
ground travel (local)	Khanty-Mansiysk to field station Mukhrino and back: c. 100 € (car or boat, as recommended by field station staff)	200 €
	from Khanty-Mansiysk north (car): 250 €	500 €
accomodation	during trip to north: 300	600 €
material for sampling	250 plastic bags; silica gel	150 €
visa costs	50 €	100 €
total		3950 €

Specific logistical needs are the visas and an export license. An import license for material of purely scientific use is not necessary for Austria. Tools for sampling and measuring are standard and will be brought along on the trip.

Distribution of work is simple – field station staff will assist in guiding us to the poplar stands, where we will make measurements and do the sampling. The genetic analysis, as well as data treatment, will be conducted after the visit in the Austrian lab (not charged to project).

6. Expected results and possible risks

Studies on continent-wide genetic distribution patterns for tree species are still rare at this scale – *Populus tremula* is one of the most widely spread tree species worldwide. The expected results will thus contribute to one of the first studies that links genetic differentiation to continent-wide patterns of growing conditions, while also forming one of the first local studies on tree clonality in this part of Russia. This should thus receive considerable scientific interest. It may lead to further studies in greater depth (i.e., with genetic markers covering larger parts of the genome, or with regard to ecologically interacting species) or in wider ranges. There are no imminent risks for failure, as the method has been applied previously, and some kind of a genetic-ecological pattern will arise from the investigations.

Results will be freely published (i.e., with an *open* access option) in international scientific journals, just as previously done by the research group. The general public will be made aware by established media contacts nationally and internationally, e.g. the web-service *Waldwissen.net* which is run by staff of the Austrian Research Centre. Data will be deposited in an online system, a requirement for publishing in high-standard journals.

- 7. Key literature:** Publications of the research group members (highlighted; B. Heinze; B. Fussi is a former member of the research group), and List of references in the research plan: De Carvalho D., Ingvarsson P.K., Joseph J., Suter L., Sedivy C., Macaya-Sanz D., Cottrell J., **Heinze B.**, Schanzer I., & Lexer C. (2010): Admixture facilitates adaptation from standing variation in the European aspen (*Populus tremula* L.), a widespread forest tree. *Molecular Ecology* 19[8], 1638-1650.
- Dickmann, D. I. & Kuzovkina, J. (2008): Poplars and Willows of the World, with Emphasis on Silviculturally Important Species. In: *Poplars and Willows in the World: Meeting the needs of society and the environment*. FAO Forest Management Division, 134 pp. FAO, Rome, Italy.
- Fussi, B.**, Bonello, J., Calleja, E., & **Heinze, B.** (2012): Combining the use of molecular techniques and archival documentary evidence to trace the origin of *Populus alba* in a Central Mediterranean archipelago. *European Journal of Forest Research* 131, 347-354.
- Fussi, B.**, Lexer, C., & **Heinze, B.** (2010): Phylogeography of *Populus alba* (L.) and *Populus tremula* (L.) in Central Europe: secondary contact and hybridisation during recolonisation from disconnected refugia. *Tree Genetics and Genomes* 6[3], 439-450.
- Heinze, B.** (2011): *Populus tremula* in Siberia: a Continental Divide Along Lake Baikal. Conservation of Forest Genetic Resources in Siberia. Proceedings of 3-rd International Conference, August 23-29, 2011, Krasnoyarsk, Russia, p. 154.
- Heinze, B. & Fussi, B.** (2008): Somatic mutations as a useful tool for studying clonal dynamics in trees. *Molecular Ecology* 17, 4779-4781.
- Ma, X.-F., Hall, D., Onge, K. R. St., Jansson, S., & Ingvarsson, P. K. (2010): Genetic differentiation, clinal variation and phenotypic associations with growth cessation across the *Populus tremula* photoperiodic pathway. *Genetics* 186[3], 1033-1044.
- van Loo, M., Joseph, J. A., **Heinze, B.**, Fay, M. F., & Lexer, C. (2008): Clonality and spatial genetic structure in *Populus x canescens* and its sympatric backcross parent *P. alba* in a Central European hybrid zone. *New Phytologist* 177[2], 506-516.
- von Wühlisch, G. (2009): EUFORGEN Technical Guidelines for genetic conservation and use of Eurasian aspen (*Populus tremula*). Bioversity International, Rome, Italy, 6 p.