Philipps Universität Marburg

Between Rear and Leading Edge

Juniper woodlands on the southern Tibetan Plateau

A high mountain forest-line-ecosystem under environmental change

Lars Opgenoorth

Philipps Universität Marburg

Between Rear and Leading Edge

Juniper woodlands on the southern Tibetan Plateau

A high mountain forest-line-ecosystem under environmental change

Dissertation
zur
Erlangung des Doktorgrades
der Naturwissenschaften
(Dr. rer. nat.)

dem
Fachbereich Biologie
der Philipps-Universität Marburg
vorgelegt von
Lars Opgenoorth
aus Düsseldorf

Marburg/Lahn 2009

Vom Fachbereich Biologie der Philipps-Universität Marburg
als Dissertation am 11.09.2009 angenommen.

Erstgutachterin: Prof. Dr. Birgit Ziegenhagen
Zweitgutachter: PD Dr. Karsten Wesche

Tag der mündlichen Prüfung am 11.09.2009

List of Papers

This thesis is based on the following papers, which are referred to in the text by the Roman numerals I-IV. In addition a research proposal to the Volkswagen foundation is included as part of the outlook. It will be referred to with the roman numeral V.

I Miehe, G., Miehe, S., Will, M., Opgenoorth, L., La Duo, Tsering Dorgeh and Liu, J.(2008): An inventory of forest relicts in the pastures of Southern Tibet (Xizang A.R.,China). Journal of Plant Ecology 194: 157–177.

II Kaiser, K., Opgenoorth, L., Schoch, W. and Miehe, G. (2009): Charcoal and fossil wood from palaeosols, sediments and artificial structures indicating Late Holocene woodland decline in southern Tibet (China). Quaternary Science Reviews 28: 1539–1554.

III Opgenoorth, L., Vendramin, G., Mao, K., Miehe, G., Miehe, S., Liepelt., S. Liu, J. and Ziegenhagen, B. (2009): Tree endurance on the Tibetan Plateau marks the world's highest known tree line of the Last Glacial Maximum. New Phytologist. In press.

IV Opgenoorth, L. (2009): Identification and characterization of microsatellite markers for *Juniperus tibetica* using next generation sequencing. Conservation Genetics Resources. In press.

V Opgenoorth, L.: „Adaptation at the limits" or "the limits of Adaptation" - Tracing abiotic stress-tolerance in Tibetan juniper trees along altitudinal and ecological gradients. Research proposal to the Volkswagen Foundation.

Paper I through IV are reprints made available with permission from Springer (I, IV), Elsevier (II), and Wiley-Blackwell (III).

Contents

Foreword

The framing of this dissertation comes in a time of severe makeup and essentially amalgamation of the different fields of paleoecology, biogeography, conservation genetics and eventually evolutionary biology mainly due to the recent revolutionary advances in sequencing technology but also due to important advances in our understanding of the natural processes. It is almost symbolic that these advances seem to culminate in the year of Darwin – with evolutionary processes finally earning the focus they have always deserved.

The makeup of the present thesis reflects this recent development only in parts: The charcoal analytical study (Paper II) as well as the phylogeographic study (Paper III) of this work, were designed in a “pre-next generation sequencing technology” context. Both studies would today have been designed differently. The charcoal analytical approach would be escorted or even replaced in large parts by shotgun sequencing approaches that utilize ancient DNA. The advantages of this approach are the wider distribution of DNA in different archives such as glaciers, permafrost and palaeosols, their superior power to determine taxa on higher levels, and in case of good conservation, their potential use in evolutionary contexts such as deep phylogeographies. Our first approaches using this technology are currently underway: Pleistocene and Holocene soil samples from Paper II have been successfully screened for aDNA recently in the lab of Eske Willerslev.

Phylogeographic studies on the other hand will change to the effect that the data sets will increase drastically. While it seemed quite advanced in the beginning of my study to sequence the whole set of our samples instead of using RFLP’s (as was anticipated in the study proposal) it already shows that such single locus data sets can hardly be published anymore. One reason is that it is so much easier to produce larger data sets now. The essential reason however is that multi-locus analysis from different genomes – or eventually whole genomes - make the analysis much more reliable and enable us to implement more complex analytical approaches such as coalescent simulation. These approaches bring us as close as ever to being able to check a priori hypothesis in a historical context – much like in the fields of experimental science. Nevertheless, even (quasi-) experimental research approaches need good background information for hypothesis building and testing.

The present study lies in the tradition of expedition science in the remoter places of this planet and is intended to provide such background information on the forest history of the Tibetan Plateau. It also intends to give an outlook how hypotheses based on this groundwork can be tested in future experimental designs. In this respect, I take the freedom to include a research proposal to the Volkswagen Foundation as final part of this thesis.

On a more personal note, the makeup of this thesis also reflects my scientific “evolution” with the two first papers reflecting my education in physical Geography and the last two papers as well as the research proposal reflecting my switch to molecular ecology.

1. Introduction

Forests are one of the most successful land biomes on Earth – currently covering about 30% of the earth's land surface despite heavy anthropogenic impact (FAO 2006). Their competitive advantage is largely indebted to the trees' large root systems, large leave-area coverage and their longevity that enable them to effectively compete for water, nutrients, and sunlight. Forests fail and have failed to compete however, in areas were the recurrent climate is too harsh to sustain the trees' large woody life-forms, or in some cases, where the immediate environment is too extreme for tree growth. Thus, ever since woody life forms have evolved, forest histories as a whole have been largely linked to (extreme) climatic oscillations.

The most pronounced climatic thresholds for tree growth are water stress and cold stress. The former is usually responsible for lower timberlines and the latter for arctic and alpine timberlines (e.g. Arno & Hammerly, 1984; Stevens & Fox, 1991). It has long been known however, that not winter minimum temperatures steer arctic and alpine timberlines but rather limitations of summer warmth, or erratic frost events, such that trees cannot complete their annual life cycle to reach frost hardiness (Arno & Hammerly, 1984). Exact numbers for these limiting factors are not known and probably do not exist. Especially water stress has too many variables (precipitation, soil texture, potential evapotranspiration …) for being sufficiently broken down in one number. However, for cold limits a general linkage between a mean air temperature of 5.5 to 7.5°C during the growing season has been proposed (e.g. Körner 1998, 2003;).

The strong linkage between forest histories and climatic thresholds or (extreme) climatic oscillations has led to a large body of literature specifically regarding the Pleistocene climatic oscillations (e.g. Frenzel, B., & Troll 1952;Frenzel 2000;Hewitt 1999;Hewitt 2004;Huntley & Birks 1983;Petit et al. 2003;Petit et al. 2002). These studies often aim at both, understanding the forest histories as well as using these as proxy for characterizing past climatic changes. In combination these insights are then used to predict future vegetation trends in times of global climate change. This type of research was first started and dominated by pollen analysis, and the analysis of macrofossils, as these methods are powerful in detecting general vegetational trends on a landscape basis. For a time the emerging field of phylogeography was happy to confirm the general patterns put forward by the traditional methods of paleoecology as this at the same time confirmed the functioning of the new method. However, in recent years it has increasingly been shown that pollen and macrofossil analysis are prone to overlooking microrefugia (thus termed cryptic refugia) (e.g. Willis et al 2000;Lister & Stewart 2001). Though of small population sizes, these microrefugia could have had significant input on preserving essential parts of the gene pools and on contributing to demographic expansions after glacial oscillations. Thus, even though the reconstruction of past forest histories and deduction of climatic scenarios probably produced correct trends on a regional to continental level, their power on a landscape to local level should be taken with caution. This is especially clear when considering the mentioned complexity of climatic factors for the existence of tree and forest lines. Simply equating past occurrence of trees or certain tree taxa with temperature and precipitation regimes is probably misguided on smaller geographic scales.

A second line of bias exists in large parts of the paleoecological literature and has in my opinion not received enough attention yet: human impact. At latest with the onset of the Neolithic Revolution the direct link between forest histories and climatic oscillations has increasingly been superimposed by human impact. Though no exact data exist, it has been estimated that per of today, up to half the world's original (post glacial) forest cover has been replaced by anthroposcapes (Loh et al., 1998). This can seriously bias any inference or prediction made on the basis of forest (or more generally vegetation) history. This is especially the case in areas where tree growth approaches its environmental limits, as anthropogenic impact does not need to be very strong to effectively eliminate tree growth there. However, the potential of past societies to change their environments often has been, and still is, under-estimated, even though it does not take anything else than the use of fire and subsequent intensive cattle herding or agricultural use to persistently change fragile ecosystems. Proxies such as pollen, micro- and macrofossils, sediments, geomorphic deposits etc. can easily help document vegetation change but unfortunately they seldom elucidate what the driving forces for these changes were – as past desiccation and desertification can produce similar signals.

The present thesis is an attempt to shed light on the forest history of the southern Tibetan Plateau as an example for high mountain research. Like few other places, high mountain areas could have a huge potential for cryptic refugia due to their heterogeneous topographies, and steep elevational gradients. Furthermore, the patchy, and in parts extreme environments could make them especially interesting for evolutionary research as well - for extreme environments may also provoke extreme adaptations – or even speciation events. Likewise, the existence of old microrefugia could mean that a large pool of specific genetic variation has been preserved.

Unfortunately often very little is known about the paleoecology of high mountain areas as they tend to be more difficult to access. Therefore paleoecological reconstructions in these areas are often based on few case studies that are used to explain highly patchy environments. Thus the biases outlined above are especially relevant in high mountain areas.

The southern Tibetan Plateau has largely been described as a high mountain semi desert or alpine steppe. The occurrence of forest relics and woodlands has long been overlooked, as has the heavy anthropo-zoogenic impact on this environment (Paper I). Consequently, past anthropo-zoogenic disturbances have largely been ignored in paleoecological, and pa-

leaoclimatological studies (Miehe et al., 2008; Kaiser et al., 2009b). At least some of the conclusions put forward in these works are thus potentially misguided.
Likewise, despite extremely little information – both on temporal as well as spatial scales – it has been preconceived that the forests of the Tibetan Plateau extirpated during the Last Glacial maximum and survived in vaguely described refugia in southeastern Asia (Tang & Shen 1996). This cannot surprise given the fact that it has only been recently that the dispute about an ice-shield covering all of the Tibetan Plateau was finally settled (Seong et al., 2008).

With the combination of macrofossil and genetic analysis and the focus on the keystone *Juniperus tibetica* species complex I hoped to be able to significantly contribute to the knowledge on the forest history of the southern Tibetan Plateau and to be able to make generalizations about the potential importance of high mountain areas for paleoecological and evolutionary research in general. More specifically the aim was to

1. Map existing forest remains and single tree stands of the chosen model taxa: *Juniperus tibetica* species complex. (Paper I)
2. Track macrofossils and charcoals as direct proof for microrefugia and for dating former forest and tree existence and fragmentation on the southern Tibetan Plateau. (Paper II)
3. Analyze the phylogeography of the model organism(s) and determine locations of potential refugia and potential postglacial recolonization routes. (Paper III)
4. Describe genetic makeup and past gene flow of the remaining forests for evaluation of the forests vitality. (Paper III)
5. Develop microsatellites as tools for the analysis of recurrent gene flow and nuclear genetic variation in order to outline conservation approaches. (Paper IV)
6. Outline future research goals in an evolutionary perspective. (Research Proposal V)

2. Results and Discussion

2.1 The forest relics in the pastures of southern Tibet (Xizang A.R.,China) – Paper I

Paper I represents the first thorough recognition of the existence of juniper forest and woodland relics on the southern Tibetan Plateau. In it, the current extension of the juniper forests was mapped, and the individual stands characterized. Due to the extreme fragmentation of the Tibetan forests and woodlands, and their limited sizes a satellite-based approach was not feasible. Instead, information were collected in person, through interviews, and by extracting information from written historical documents. Figure 1 in Paper I summarizes today's known distribution of juniper forests on the central southern Tibetan Plateau. There it can be seen that the woodlands and forests – though highly scattered and fragmented – have a wide distribution range throughout the southern Tibetan Plateau. In the descriptions of the individual stands it becomes evident, that the forests, woodlands and single trees occur on normal sites, usually south-facing slopes without water-surplus. Furthermore, the documentation of seedlings in 23 out of 28 populations with such records, demonstrates the potential viability of these stands (table 1 in Paper I). The 23 populations with rejuvenation occur along the whole distribution range – despite decreasing precipitation sums towards the west. As a consequence, we concede in the study, that the existence of these stands poses a contradiction to the formerly held notion of a natural treeless environment.
However, the paper also acknowledges that most stands are over-aged and show few young trees in the range of 5-20 years despite sometimes heavy fructification. This striking discrepancy of fruiting trees and the scarceness of seedlings has been observed in other juniper species worldwide (e.g. *J. sabina* in Mongolia, Wesche et al., 2005; and *J. communis* in Germany, Verheyen et al., 2009) and has been linked with a limitation in viable seed production recently at least for *Juniperus communis* in Europe (Verheyen et al., 2009). Paper I presents similarly low percentages of viable seeds for the Tibetan junipers as evident by Tetrazolium tests (less than two percent viable seeds for the Reting forest) and germination trails (less than one percent germinating seeds for the Reting forest). The reason for the low amount of viable seeds however is not well understood yet. In the same study mentioned above Verheyen et al. propose that high temperatures and nitrogen deposits are correlated with the low seed viability. In 2000 Garcia et al. (Garcia et al., 2000) tested whether low seed viability is a way of protection against seed predation but found no evidence for this at least for predation by *Apodemus sylvaticus* (Garcia et al., 2000).

Besides the low seed viabilities, numerous studies could convincingly show the strong impact grazing pressure has for poor regeneration success in junipers, even though junipers need raw soil as habitat (Ward, 1973; Ward, 1982; Fitter & Jennings, 1975; Gilbert, 1980;

Verheyen et al., 2009). The fencing trails in Paper I also showed this negative impact, even though only large herbivores were successfully fenced thus that Pica and other small mammals were likely reasons why most of the offspring did not survive the first winter.

Nevertheless, reforestation trails above Lhasa showed that different juniper species have survived without irrigation since 1999 and the fact that the Tibetan junipers grow to more than 1000 years of age means that without anthropo-zoogenic pressure only rare successful rejuvenation events were necessary for sustaining viable populations.

2.2 Late Holocene woodland decline in southern Tibet (China) – Paper II

For paleoecological analysis two charcoal proxies are generally of key interest: Charcoal accumulation rate (CHAR) of microfossils in sediments, such as peats or lacustrines, as a measure for regional – or biome-typical fire frequencies, and charcoal fragments larger than 400 µm from soils or palaeosols as a tool to reconstruct the local woody palaeovegetation (Lynch et al., 2004; Carcaillet, 2007). Ideally both types of archives are analyzed together. Unfortunately until now only very little information is available for both proxies on the Tibetan Plateau. The few charcoal accumulation rates available from pollen records show fire activity throughout the Holocene without proper identification of distinct 'fire periods' however (van Leeuwen in La Duo, 2008; Schlütz et al., 2007; Kaiser et al., 2008). Part of the problem is that all but one of the available pollen records in the area contain hiatuses. In this light it was the aim of Paper II to make use of charcoal findings from existing geomorphological and archeological studies (Kaiser et al., 2006b; Kaiser et al., 2009a; Kaiser, 2004), and to find new archives along the collection route of Paper III to help reconstruct the Holocene forest history of southern Tibet.

All together 46 sampling sites along an 800 km range in elevations between 3500-4700 m a.s.l. were found. From these, 53 botanical spectra could be obtained comprising 27 taxa. Resembling today's tree spectra, the most dominant phanerophyte taxa were *Juniperus*, *Hippophae*, *Salix*, and *Betula*. In addition, *Populus*, *Pinus*, *Quercus*, *Taxus* and *Pseudotsuga* were found. The fossil taxa were arranged into 'ecological groups' according to probable present-day equivalents (see Table 1, Paper II) including upland tree species, wetland tree and shrub species, shrub species of the forest understory and above treeline, and cultivated species.

The spectrum of 55 radiocarbon ages of charcoals indicates a discontinuous burning record that culminated in the Late Holocene (Table 2, Paper II). The fact that charcoal and fossil wood prove a Late Holocene existence of woodlands in today unwooded areas indicates a Late Holocene vegetation change from woodlands to the present desertic pastures. The existing archeological evidence for increasing importance of agrarian economies in southern and south-eastern Tibet for the same time (Aldenderfer & Zhang, 2004; Fu et al., 2000; Kaiser et al., 2006a; Kaiser et al., 2009a) suggest that these vegetation changes were triggered by human induced woodland burning and subsequent grazing pressure. This interpretation is in line with similar conclusions drawn by Meyer et al (Meyer et al., 2009) for the adjacent northwest Bhutan, and conclusions by Schlütz and Zech (Schlütz & Zech, 2004) in a similar environment in Nepal.

Nevertheless, future high resolution fire frequency studies are needed in order to reliably correlate charcoal peaks with anthropogenic impact. Our study showed that charcoal frequencies in palaeosols and various other sediments and artificial structures is promising for such high resolution studies.

2.3 Tree endurance on the Tibetan Plateau marks the world's highest known tree line of the Last Glacial Maximum – Paper III

So far the existence of the south Tibetan woodlands and forest islands had been explained by postglacial recolonizations out of southern and southeastern refugia along the large river gorges (e.g. Tang & Shen, 1996). Given that heavy fragmentation of the juniper woodlands dates back to the Late Holocene, and given the longevity of junipers only relatively few generations would have been available for postglacial recolonization until fragmentation processes halted the expansion (see Paper II). Considering the observed reproductive inefficiency of *Juniperus* (see Paper I) this short amount of time between potential recolonization, spatial expansion until heavy fragmentation set in seems implausible. Thus, it was the aim of Paper III to elucidate this potential contradiction by testing different hypothesis that could explain the history of the current day juniper woodlands: i) glacial extirpation and postglacial recolonization out of southeastern refugia ii) glacial extirpation and postglacial recolonization out of Himalayan and southeastern refugia and iii) glacial in situ survival and postglacial spatial expansion from decentralized microrefugia.

It was originally anticipated to use *Juniperus tibetica* as model organism for this research. It turned out that this species forms a species complex with 4 other closely related juniper species, namely *J. convallium*, *J. saltuaria*, *J. indica*, and *J. microsperma* that due to hybridization or incomplete lineage sorting could not be properly delineated in most populations. For this purpose we included the whole species complex in the analysis. 590 trees out of 102 populations were sequenced in three polymorphic chloroplast regions. 62 haplotypes were derived of which 40 were restricted to single populations. These private haplotypes were evenly spread along the distribution range of the species complex with no differences between plateau edges and the plateau platform. Given the slow mutation rates generally observed in the chloroplast genomes (e.g. $1.2\text{-}1.7 \times 10^{-9}$ substitutions per site per year for cpDNA in Graur & Li, 2000) it is highly unlikely that these private haplotypes occurred during postglacial recolonization. Instead they are a clear indication that the populations endured the LGM in microrefugia along the entire distribution range. Comparable mutation rates, levels of diversity and differentiation in the same chloroplast regions were found for *Picea glauca* in Alaska and were also taken as evidence for the existence of LGM refugia in

Alaska (Anderson et al., 2006).

Furthermore the phylogeographic structure found in our study clearly showed a split between Himalayan haplotypes and plateau haplotypes proving that Himalayan populations did not significantly contribute to the current juniper forests and woodlands on the Plateau.

As the valley bottoms on the southern Tibetan Plateau lie between 3500-4400 m a.s.l. the microrefugia on the Tibetan Plateau mark the world's highest known tree lines of the Last Glacial Maximum. As such, they are an impressive confirmation for the large potential of high mountain areas to sustain cryptic refugia and that an increased focus on these areas could be key to retelling the story of glacial survival and postglacial re-expansion of numerous plant and animal taxa. Furthermore we suggest in our study, that microrefugia in high mountain areas can have major evolutionary and conservation significance. In 2005 Hampe and Petit proposed (Hampe & Petit, 2005) that rear edge populations are often disproportionately important for the survival and evolution of biota due to the longevity of their gene pools. Rear and leading edge populations in high mountains have a special characteristic in that they are very close to each other – separated only by an orographic gradient but very limited distance. As a consequence – gene flow is possible between them. Given that rear and leading edge populations are considered to have different adaptational potentials (e.g. drought in rear edges and cold at the leading edges Hampe & Petit, 2005) orographic rear edge populations potentially combine the complete spectrum of adaptation.

Finally, when the mutation rates observed in our data are put into perspective, it is even likely that the genetic pattern observed on the Tibetan Plateau is of late Tertiary/ early Pleistocene origin potentially dating back to latest stages of the uplift of the Himalayas and the Tibetan Plateau. In recent years numerous studies worldwide have proposed old origins of chloroplast patterns – e.g. a Miocene origin of genetic pattern of Mediterranean *Quercus suber* (Hampe & Petit, 2007) and a Tertiary origin of the genetic patterns of *Quercus lobata* in California (Grivet et al., 2006).

2.4 Identification and characterization of microsatellite marker using next generation sequencing - Paper IV

The use of cpDNA marker in Paper III was appropriate for the phylogeographic approach, given that no prior knowledge was available for the Tibetan junipers. However, a whole range of future topics cannot be approached sufficiently with this marker system alone, as for example estimating nuclear genetic diversity and specifying recurrent gene-flow for conservation strategies, determining the different taxonomic demes using structure, using a coalescent approach to better understand the demographic history of the species and of individual populations in order to gain a good neutral model for subsequent association mappings. For these purposes microsatellite markers were developed in Paper IV. The initial approach was to try to use SSRs developed in *Juniperus communis* (Michalczyk et al., 2006) and transfer them to the Tibetan juniper species. Unfortunately this attempt largely failed due to the poor transferability of marker among the species and difficulties with the original primer sequences. This poor transferability has been observed in other transfer tests involving Cupressaceae. For example, in one recent study two out of 800 candidate genes taken from *Pinus taeda* were successfully resequenced in different *Juniperus* and *Cupressus* species (CRIEC project, unpublished).

As a consequence I followed a new approach based on next generation sequencing technology proposed by Abdelkrim et al (2009) and combined it with a cost-efficient microsatellite labeling approach introduced by Schuelke (2000). In short, one lane of a 454 Titanium run was used for a shotgun sequencing approach on genomic DNA from a *Juniperus tibetica* sample. This yielded 67,782 reads at an average read length of 339 bp, with a mode at 419 bp. The MSATCOMMANDER software (Faircloth, 2008) was used to screen these reads for microsatellite motifs and to develop primers in the flanking region. With the cost-efficient labeling system proposed by Schuelke it was possible to screen 50 primer pairs on a set of 16 individuals from 8 populations. Ten of the 50 primer pairs produced polymorphic bands (Paper IV) and are thus ready for use. Besides the mentioned research incentives these resources will be key for developing a neutral model for the research proposal reprinted as Paper V.

3. Conclusions

Our studies on the juniper forests and woodlands of the southern Tibetan Plateau change the story line of the area's forest history in large parts.

1. Juniper forest patches and/or woodlands endured the Last Glacial Maximum on the southern Tibetan Plateau.
2. The Himalayan populations did not significantly contribute to the current forests on the Tibetan Plateau.
3. Postglacial demographic and probably spatial expansion took place from the decentralised microrefugia.
4. Heavy fragmentation of the forests/ woodlands started towards the Late Holocene and was probably caused by human impact.
5. The occurrence and distribution of today's forest and woodland remains as well as the outlined forest history indicate that forestation of larger parts of the southern Tibetan Plateau are still possible under current climatic conditions.
6. Genetic resources are in place to assess recurrent gene flow, and determine the nuclear genetic diversity for setting up conservation strategies as well as producing a neutral model for further evolutionary studies.

With these studies we could confirm the existence and importance of cryptic refugia that in recent years have received growing attention worldwide. Furthermore, we could show, that high mountain areas are especially suited for microrefugia due to their heterogeneous topographies. I therefore hope that our work will encourage further studies in such areas. Finally I hope that this study sheds light on the importance of (historic) human impact on (fragile) ecosystems and the potential pitfalls this creates in understanding and interpreting vegetation and climatic history.

4. Outlook

Many interesting questions were raised in the course and as a result of our research on the juniper forests of the southern Tibetan Plateau that could not be answered in the process of this work. Other findings need to be confirmed by additional research. Some of these topics relate only to the biology of the species complex itself, are relevant to the regional natural history of the southern Tibetan Plateau, or for direct conservation measures intended at preserving and reforesting the Tibetan juniper forests. A few examples for such future research incentives are: Large scale fencing and rejuvenation trials as proposed in Paper I in order to assess the large scale potential for reforestation of the Tibetan Plateau today and to better understand the regeneration strategy of junipers; build up of a regional CHAR database and implementation of a shotgun sequencing approach targeting aDNA in sediment cores to more completely reconstruct the natural history of the Tibetan Plateau (Paper II); produce coalescent based phylogeographies with co-occurring forest species as for example mycorrhiza associated with the junipers or insect taxa to construct a natural model for the ecosystem as a whole (Paper III); utilization of SSRs to assess the recurrent gene flow and the nuclear genetic diversity of the remaining juniper populations for outlining conservation strategies (Paper IV).

Other questions derived from our research are of broader evolutionary interest. The research proposal included in this dissertation as ‘Research Proposal V’ focuses on these latter questions:

4.1 „Adaptation at the limits” or “the limits of Adaptation” - Tracing abiotic stress tolerance in Tibetan juniper trees along altitudinal and ecological gradients - Research Proposal V

The extreme environmental conditions on the Tibetan Plateau, especially coldstress towards the alpine treeline, drought towards the lower treeline, and one of the highest UV radiation levels in the world throughout the Tibetan Plateau, must have provoked extreme adaptations in organisms living there. As a consequence association studies aiming at detecting genes responsible for such adaptations are extremely promising in this setting. Furthermore, in Paper III we proposed that orographic rear edge populations could harbor a specific evolutionary potential based on the fact that as stable rear edge populations they have survived at least one or probably several glacial/interglacial cycles, and on top of that potentially carry the complete adaptational spectrum of rear and leading edge populations. Finally, since we have indications that the *Juniperus tibetica* species complex is a hybrid complex, questions regarding the role of hybridization as a tool for extended adaptational potential arise. Did environmental stress and adaptation to it favor speciation events in the Tibetan junipers or conversely trigger adaptive introgression?

Despite their exceptional importance as key species in landscapes, trees have been dealt with comparatively little regarding the detection of complex traits. This is partly due to the fact that as long-lived and sessile organisms trees are usually considered difficult experimental organisms (Neale & Savolainen 2004). In addition the genomes of tree species are often extremely large, especially those of conifers. Therefore sequencing essential parts of their genomes has been not feasible so far (Neale & Savolainen 2004). The recent advances in sequencing technology now enable us to perform such studies. The method of choice for the time being for detecting adaptively relevant genes is association mapping (e.g. Neale and Savolainen 2004). It combines genome-wide sampling with traditional population genetic and quantitative genetic approaches to understanding evolution (Gonzalez-Martinez et al., 2006). The basis for the study proposed in Research Proposal V would be twofold: First, screening of candidate genes available from INRA Bordeaux, David Neale and Giovanni Vendramin derived from physiological studies and other genomic studies; and second, the build-up of an EST database by means of high-throughput sequencing. The latter is also intended for the comparison of genes that are responsible for speciation, and genes associated with adaptations to the key traits mentioned above.

The specific aims of the research proposal are as follows:

1. Identify and characterize genes for drought-, cold- and UV-radiation-tolerance in *Juniperus tibetica, Juniperus saltuaria* and *Juniperus convallium.*
2. Identify polymorphisms at the selected candidate genes.
3. Compare the diversity of adaptive traits of orographic rear edge populations with orographic leading edge populations and with latitudinal rear edge populations.
4. Compare patterns of nucleotide diversity at candidate genes in the tree species in allopatric and sympatric situations.
5. Characterize genes relevant for speciation (performed by Dr. J. Liu based partly on his sequencing and on the pyrosequencing performed in this project) and compare those with genes found to be responsible for key adaptations.

5. Deutsche Zusammenfassung

Aufgrund der ökosystemaren Bedeutung von Wäldern und ihrer engen Kopplung an das Klima setzen sich weite Teile der Paläoökologie mit der Geschichte der Wälder im Zuge pleistozäner Klimaoszillationen auseinander. Weltweit konnten so die Grundzüge der Vegetations- und Waldgeschichte mit Hilfe der Pollenanalyse, Analyse von Makrofossilien und zuletzt der Phylogeographie erkundet werden. Allerdings kamen in den letzten Jahren vermehrt Hinweise auf, die zeigen, dass Mikrorefugien und ihre Bedeutung für die Konservierung von genetischem Material, sowie ihrer Bedeutung bei der postglazialen Wiederbesiedlung lange Zeit übersehen wurden. Diese übersehenen Refugien werden Kryptische Refugien genannt. Ausserdem wurde in den letzten Jahren deutlich, dass der z.T. sehr gravierende und vor allen Dingen frühe menschliche Einfluss auf Vegetations- und insbesondere Waldentwicklung in der Paläoökologie keine angemessene Berücksichtigung fand. Daher kam und kommt es zu Mißinterpretationen bei der Ableitung von Klimaparametern und der Prognose zukünftiger Vegetationsentwicklung.

Die hier vorliegende kumulative Dissertation bearbeitet die Waldgeschichte des Tibetischen Plateaus vor diesem Hintergrund und postuliert eine besondere Bedeutung von Hochgebirgslandschaften für den Erhalt von Mikrorefugien. In insgesamt 4 Artikeln werden sowohl klassiche paläoökologische Methoden sowie molekulargenetische Methoden verwendet, um die Waldgeschichte Südtibets am Beispiel der Wacholderwälder zu rekonstruieren. Da das Tibetische Plateau heute in weiten Teilen durch alpine Steppen, Hochgebirgshalbwüsten und Weideländern geprägt ist und neben Auwäldern nur vereinzelt Waldinseln und Einzelbäume auf Normalstandorten auftreten, wurde die Waldgeschichte des Plateaus lange Zeit übersehen.

Im ersten Teil dieser Arbeit (Paper I) wurden die aktuell bzw. historisch bis in die 1960er Jahre hinein existierenden Wacholderwälder kartiert und charakterisiert. Demnach sind in weiten Teilen des südlichen Plateaus Waldrelikte vorhanden. Die natürliche Verjüngung an vielen dieser Standorte, sowie Versuche in Baumschulen zeigen, dass das Waldpotential auch unter derzeitigen Klimabedingungen gegeben ist. Ungeklärt bleibt hingegen die Ursache für die extreme Diskrepanz zwischen der hohen Anzahl an Beerenzapfen und dem geringen Anteil an vitalen Embryonen. Die Tatsache, dass dieses Phänomen in unterschiedlichen Wacholderarten unter unterschiedlichen klimatischen Bedingungen weltweit beobachtet wurde macht einen Zusammenhang mit klimatischen Stress allerdings unwahrscheinlich.

In Paper II wird mit Hilfe von Holzkohleanalysen gezeigt, dass bis ins späte Holozän die Wacholderwälder Südtibets weiter verbreitet waren. Ihre Reduzierung auf das heutige Maß wird mit wachsendem menschlichen Einfluss einschliesslich der Yakviehhaltung im Zusammenspiel mit klimatischer Austrocknung gedeutet. Um diese Befunde zu bekräftigen ist allerdings in Zukunft die Erarbeitung hochauflösender Holzkohledatenbanken notwendig. Das dazu nötige anthrakologische Potential in den Geoarchiven Südtibets konnte mit dieser Arbeit aufgezeigt werden.

Im Dritten Artikel (Paper III) wird mit Hilfe molekulargenetischer Methoden gezeigt, dass entgegen bisheriger Annahmen die Wacholderwälder Südtibets im Letzten Glazialen Maximum auf dem Tibetischen Plateau in Mikrorefugien überdauerten. Auch konnte gezeigt werden, dass die himalayischen Wacholderbestände nicht wesentlich zu einer postglazialen Wiederbesiedlung des Plateaus beigetragen haben. Die hohe Anzahl privater Haplotypen auf dem Plateau sowie die gewöhnlich geringen Mutationsraten im Chloroplastengenom machen sogar eine Entstehung der beobachteten genetischen Muster im späten Tertiär plausibel. Darüber hinaus demonstrieren diese zahlreichen dezentralen Mikrorefugien, sowie die hohe Anzahl endemischer Haplotypen das Potential von Hochgebirgslandschaften mit ihren heterogenen Topographien für den Erhalt genetischen Ressourcen.

Das rein methodische Paper IV liefert Grundlagen für die weitere Erforschung dieser genetischen Ressourcen und ihrer Verteilung in der Landschaft. Mit Hilfe von Hoch-Durchsatz-Sequenziermethoden wurden Mikrosatelliten entwickelt, die bei der Analyse von aktuellen Genflüssen, sowie nukleärer Diversität zum Einsatz kommen sollen. Darüber hinaus sind sie die Grundlage um die in Paper V und im Kapitel Outlook skizzierten Forschungsvorhaben zu realisieren.

6. Acknowledgements

As can be seen from the papers this work was done in cooperation with many different people and I would like to thank all of my co-authors for their input to this research and the many interesting discussions we had. I hope that some joint articles will follow in the future. Of course there is an even greater number of people ‘behind the scenes’, without whom this thesis would not have been possible. The first two that I want to mention are Birgit Ziegenhagen and Georg Miehe whose wholehearted support helped me in so many ways. Despite german (Birgit) and chinese (Georg) bureaucracies - please always keep your scientific enthusiasm alive as it is and keep on infecting other people with it.

Second, I would like to thank Tina Mengel who sees people coming and going like in Grand Central Station and still seems to enjoy to get everyone going with the lab essentials. Thank you for getting me started (and finished)!

The two trips to the Tibetan Plateau would not have been the same without all our Tibetan and Chinese collegues and friends. Sorry for all my cultural misteps, and thank you for your support and patience. Likewise, the second trip would not have been the same without Sabine Miehe. Thank you for your insights into science, Tibet and humanity in general. It was a pleasure walking the Himalayas and the Plateau with you. And Georg, always again!

I would like to thank the whole Ziegenhagen crew that was made up of many people over the years for support, laughs and a home in the biology department. Of those I would especially like to thank Inga, Eva and Marc for everything except for the fact, that you left ahead of me. Marc, I think we still have to play the ultimate match. I will meet you after the disputation. The winner may take on Sascha and Eike.

A big thank you also to my second home in the department of biology, the hunters, road kill cooks and followers of other ‘manly’ rituals in the AG Brandl. Staying in your part of the building was a pleasure and your input was very helpful.

I would like to thank the many collegues world-wide for their support and friendship, most notably Beppe Vendramin and his crew from GeneExpress in Florence, and the Evoltree network of Excellence for scientific input as well as necessary funds to receive all the trainings. The major parts of the funds for this project came from the German Research Council and the German Academic Exchange Program. This support was highly appreciated.

Finally, thanks to the phd commission, namely Birgit Ziegenhagen, Karsten Wesche, Roland Brandl and Michael Bölker, to take the time to read through this little book and thus contribute to finalize this phd.

Most of all however, thank you to my family and friends on the other side of the forest for original enthusiam about my Tibet travels and subsequent patience with the many hours spent in the lab and in front of the computer. I hope I have not mutated to some sort of weird alien in your eyes.

Bibliography

1. Abdelkrim, J., Robertson, B., Stanton, J. A., & Gemmell, N. J. (2009) Fast, cost-effective development of species specific microsatellite markers by genomic sequencing. Bio Techniques, 46, 185-192.
2. Aldenderfer, M. & Zhang, Y. N. (2004) The prehistory of the Tibetan plateau to the seventh century AD: Perspectives and research from China and the West since 1950. Journal of World Prehistory, 18, 1-55.
3. Anderson, L. L., Hu, F. S., Nelson, D. M., Petit, R. J., & Paige, K. N. (2006) Ice-age endurance: DNA evidence of a white spruce refugium in Alaska. Proceedings of the National Academy of Sciences of the United States of America, 103, 12447-12450.
4. Arno, S. F. & Hammerly, R. P. (1984) Timberline. Mountain and Arctic Forest Frontiers. The Mountaineers, Seattle.
5. Carcaillet, C. (2007) Charred Particle Analysis. Encyclopedia of Quaternary Science (ed. by S.A.Elias), pp. 1582-1593. Elsevier.
6. Faircloth, B. C. (2008) MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. Molecular Ecology Resources, 8, 92-94.
7. FAO (2006) Global Forest Resources Assessment 2005. (ed. by FAO), pp. 1-320. Food and Agriculture Organization of the United Nations, Rome.
8. Fitter, A. H. & Jennings, R. D. (1975) Effects of Sheep Grazing on Growth and Survival of Seedling Junipers (Juniperus communis L). Journal of Applied Ecology, 12, 637-642.
9. Frenzel, B. (2000) Flora and Vegetation during the Quaternary. Progress in Botany, 61, 303-334.
10. Frenzel, B. & Troll, C. (1952) Die Vegetationszonen des nördlichen Eurasiens während der letzten Eiszeit. Eiszeitalter und Gegenwart, 2, 154-167.
11. Fu, D. X., Xu, T. W., & Feng, Z. Y. (2000) The ancient carbonized barley (Hordeum vulgare L., Var. nudum) kernel discovered in the middle of Yalu Tsanypo river basin in Tibet. Southwest China Journal of Agricultural Sciences, 13, 38-41.
12. Garcia, D., Gomez, J. M., Zamora, R., & Hodar, J. A. (2000) Do empty Juniperus communis seeds defend filled seeds against predation by Apodemus sylvaticus? Ecoscience, 7, 214-221.
13. Gilbert, O. L. (1980) Juniper in Upper Teesdale. Journal of Ecology, 68, 1013-1024.
14. Gonzalez-Martinez, S. C., Ersoz, E., Brown, G. R., Wheeler, N. C., & Neale, D. B. (2006) DNA sequence variation and selection of tag single-nucleotide polymorphisms at candidate genes for drought-stress response in Pinus taeda L. Genetics, 172, 1915-1926.
15. Graur, D. & Li, W.-H. (2000) Fundamentals of Molecular Evolution, 2nd edn. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.
16. Grivet, D., Deguilloux, M. F., Petit, R. J., & Sork, V. L. (2006) Contrasting patterns of

historical colonization in white oaks (Quercus spp.) in California and Europe. Molecular Ecology, 15, 4085-4093.

17. Hampe, A. & Petit, R. J. (2007) Ever deeper phylogeographies: trees retain the genetic imprint of Tertiary plate tectonics. Molecular Ecology, 16, 5113-5114.
18. Hampe, A. & Petit, R. J. (2005) Conserving biodiversity under climate change: the rear edge matters. Ecology Letters, 8, 461-467.
19. Hewitt, G. M. (1999) Post-glacial re-colonization of European biota. Biological Journal of the Linnean Society, 68, 87-112.
20. Hewitt, G. M. (2004) Genetic consequences of climatic oscillations in the Quaternary. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences, 359, 183-195.
21. Huntley, B. & Birks, H. J. B. (1983) An Atlas of Past and Present Pollen Maps for Europe: 0-13000 Years Ago. Cambridge University Press, Cambridge.
22. Kaiser, K. (2004) Pedogeomorphological transect studies in Tibet: implications for landscape history and present-day dynamics. Prace Geograficzne, 200, 147-165.
23. Kaiser, K., Lai, Z. P., Schneider, B., Reudenbach, C., Miehe, G., & Bruckner, H. (2009a) Stratigraphy and palaeoenvironmental implications of Pleistocene and Holocene aeolian sediments in the Lhasa area, southern Tibet (China). Palaeogeography Palaeoclimatology Palaeoecology, 271, 329-342.
24. Kaiser, K., Miche, G., Schoch, W. H., Zander, A., & Schlutz, F. (2006) Relief, soil and lost forests: Late Holocene environmental changes in southern Tibet under human impact. Holocene Landscape Development and Geoarchaeological Research, 142, 149-173.
25. Kaiser, K., Opgenoorth, L., Schoch, W. H., & Miehe, G. (2009b) Charcoal and fossil wood from palaeosols, sediments and artificial structures indicating Late Holocene woodland decline in southern Tibet (China). Quaternary Science Reviews, 28, 1539-1554.
26. Knut, K., Zhongping, L., Birgit, S., Werner, H. S., Xuhui, S., Georg, M., & Helmut, B. (2008) Sediment sequences and paleosols in the Kyichu Valley, southern Tibet (China), indicating Late Quaternary environmental changes. Island Arc, 9999.
27. Körner, C. (2003) Alpine plant life: functional plant ecology of high mountain ecosystems, 2 edn. Springer, Berlin.
28. Körner, C. (1998) A re-assessment of high elevation treeline positions and their explanation. Oecologia, 115, 445-459.
29. La Duo (2008) Studies in the Ecology and Palaeoecology of the Lhasa Valley, Tibet Autonomous Region, China. Department of Biology, University of Bergen (Norway), Bergen.
30. Loh, J., Randers, J., Mac Gilivray, A., Kapos, V., Groombridge, B., & Jenkins, M. (1998) Living Planet Report 1998. WWF, Gland.
31. Lynch, J. A., Clark, J. S., & Stocks, B. J. (2004) Charcoal production, dispersal, and deposition from the Fort Providence experimental fire: interpreting fire regimes from charcoal records in boreal forests. Canadian Journal of Forest Research-Revue Canadienne

de Recherche Forestiere, 34, 1642-1656.

32. Meyer, M. C., Hofmann, C. C., Gemmell, A. M. D., Haslinger, E., Hausler, H., & Wangda, D. (2009) Holocene glacier fluctuations and migration of Neolithic yak pastoralists into the high valleys of northwest Bhutan. Quaternary Science Reviews, 28, 1217-1237.
33. Michalczyk, I. M., Sebastiani, F., Buonamici, A., Cremer, E., Mengel, C., Ziegenhagen, B., & Vendramin, G. G. (2006) Characterization of highly polymorphic nuclear microsatellite loci in Juniperus communis L. Molecular Ecology Notes, 6, 346-348.
34. Miehe, G., Miehe, S., Will, M., Opgenoorth, L., Duo, L., Dorgeh, T., & Liu, J. Q. (2008) An inventory of forest relicts in the pastures of Southern Tibet (Xizang AR, China). Plant Ecology, 194, 157-177.
35. Neale, D. B. & Savolainen, O. (2004) Association genetics of complex traits in conifers. Trends in Plant Science, 9, 325-330.
36. Opgenoorth, L., Vendramin, G. G., Mao, K., Miehe, G., Miehe, S., Liepelt, S., Liu, J. Q., & Ziegenhagen, B. (2009) Tree endurance on the Tibetan Plateau marks the world’s highest known tree line of the Last Glacial Maximum. New Phytologist. In press.
37. Petit, R. J., Aguinagalde, I., de Beaulieu, J. L., Bittkau, C., Brewer, S., Cheddadi, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Muller-Starck, G. M., mesure-Musch, B., Palme, A., Martin, J. P., Rendell, S., & Vendramin, G. G. (2003) Glacial refugia: Hotspots but not melting pots of genetic diversity. Science, 300, 1563-1565.
38. Petit, R. J., Brewer, S., Bordacs, S., Burg, K., Cheddadi, R., Coart, E., Cottrell, J., Csaikl, U. M., van Dam, B., Deans, J. D., Espinel, S., Fineschi, S., Finkeldey, R., Glaz, I., Goicoechea, P. G., Jensen, J. S., Konig, A. O., Lowe, A. J., Madsen, S. F., Matyas, G., Munro, R. C., Popescu, F., Slade, D., Tabbener, H., de Vries, S. G. M., Ziegenhagen, B., de Beaulieu, J. L., & Kremer, A. (2002) Identification of refugia and post-glacial colonisation routes of European white oaks based on chloroplast DNA and fossil pollen evidence. Forest Ecology and Management, 156, 49-74.
39. Schlütz, F., Miehe, G., & Lehmkuhl, F. (2007) Zur Geschichte des groeßten alpinen Oekosystems der Erde - Palynologische Untersuchungen zu den Kobresia-Matten SE-Tibets. Berichte der Reinhold-Tüxen Gesellschaft, 19, 23-36.
40. Schlutz, F. & Zech, W. (2004) Palynological investigations on vegetation and climate change in the Late Quaternary of Lake Rukche area, Gorkha Himal, Central Nepal. Vegetation History and Archaeobotany, 13, 81-90.
41. Schuelke, M. (2000) An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology, 18, 233-234.
42. Seong, Y. B., Owen, L. A., Bishop, M. P., Bush, A., Clendon, P., Copland, L., Finkel, R., Kamp, U., & Shroder, J. F. (2008) Quaternary glacier history of the Central Karakoram - Reply. Quaternary Science Reviews, 27, 1656-1658.
43. Stevens, G. C. & Fox, J. F. (1991) The Causes of Treeline. Annual Review of Ecology and Systematics, 22, 177-191.
44. Stewart, J. R. & Lister, A. M. (2001) Cryptic northern refugia and the origins of the mod-

ern biota . Trends in Ecology & Evolution, 16, 608-613.

45. Tang, L. Y. & Shen, C. M. (1996) Late Cenozoic vegetational history and climatic characteristics of Qinghai-Xizang Plateau. Acta Micropalaeontologica Sinica, 13, 321-337.
46. Verheyen, K., Adriaenssens, S., Gruwez, R., Michalczyk, I. M., Ward, L. K., Rosseel, Y., Van den Broeck, A., & García, D. (2009) Juniperus communis: victim of the combined action of climate warming and nitrogen deposition? Plant Biology.
47. Ward, L. K. (1973) Conservation of Juniper .1. Present Status of Juniper in Southern England. Journal of Applied Ecology, 10, 165-188.
48. Ward, L. K. (1982) The Conservation of Juniper - Longevity and Old-Age. Journal of Applied Ecology, 19, 917-928.
49. Wesche, K., Ronnenberg, K., & Hensen, I. (2005) Lack of sexual reproduction within mountain steppe populations of the clonal shrub Juniperus sabina L. in semi-arid southern Mongolia. Journal of Arid Environments, 63, 390-405.
50. Willis, K. J., Rudner, E., & Sumegi, P. (2000) The full-glacial forests of central and southeastern Europe. Quaternary Research, 53, 203-213.

Paper I

Plant Ecol
DOI 10.1007/s11258-007-9282-0

ORIGINAL PAPER

An inventory of forest relicts in the pastures of Southern Tibet (Xizang A.R., China)

**Georg Miehe · Sabine Miehe · Martin Will ·
Lars Opgenoorth · La Duo · Tsering Dorgeh ·
Jianquan Liu**

Received: 13 July 2006 / Accepted: 9 March 2007

Abstract An inventory of isolated tree stands surrounded by desert pastures in Southern Tibet (A.R. Xizang, China) revealed more than 50 sites with vigorous trees of *Juniperus convallium* Rehder & E.H. Wilson and *Juniperus tibetica* Kom and additional more than 10 records where juniper trees had been destroyed between 1959–1976. The tree stands are not restricted to any specific habitat, and occur within an area stretching 650 km westwards from the current forest border of Southern Tibet. The trees are religious landmarks of the Tibetan Buddhists. The highest trees were found at an elevation of 4,860 m. Vegetation records, rainfall correlations and temperature data collected by local climate stations and successful reforestation trials since 1999 indicate that forest relicts fragmented through human interference could regenerate if current cattle grazing and deforestation practices are halted. The drought line of *Juniperus* forests in Southern Tibet is approximately 200–250 mm/a. A first pollen diagram from Lhasa shows forest decline associated with the presence of humans since at least 4,600 yr BP. The currently degraded commons developed in the last 600 yr. To date, no findings of remains of ancient forests in the Central Tibetan Highlands of the Changtang have been reported.

Keywords China · Environmental change · Forest history · Habitat fragmentation · *Juniperus* · *Kobresia* · Tibet

Introduction

The issue of isolated *Juniperus* tree stands in arid Southern Tibet gives occasion to re-consider perceptions of the dimension of global change during the Holocene in the desert belt of the Old World. This is particularly challenging due to the fact that the reconstruction of Holocene environments is nowhere more difficult than in arid environments. While sediment, landform, pollen and molecular analyses may provide valuable information about shifts of vegetation belts and reforestation migration routes during the Holocene, it is nearly impossible to detect the human impact on environmental changes from hunters and gatherers, nomadic pastoralists or sedentary agriculturalists because archaeological findings are rare and palynological detection of human

G. Miehe (✉) · S. Miehe · M. Will · L. Opgenoorth
Faculty of Geography, University of Marburg,
Deutschhausstr. 10, Marburg 35032, Germany
e-mail: miehe@staff.uni-marburg.de

L. Duo · T. Dorgeh
Department of Biology, Tibet University Lhasa,
36 Jiangso Road, Lhasa, A.R. Xizang 850000, China

J. Liu
Northwest Institute of Plateau Biology, Chinese Academy
of Sciences, 59 Xiguan Street, Xining, Qinghai 810001,
China

indicator pollen in the area under consideration is still in its infancy. Owing to the weak regeneration ability of natural resources of pastures and forests in arid climates, human interference is more devastating than in moist climates and can easily lead to desertification. The result is that the natural vegetation is largely replaced by plant species commonly associated with arid environments and believed to reflect an arid ecosystem. While the primacy of desiccation since 5000 BP is widely accepted for the Sahara, where it changed *Acacia* woodlands and lakes to hyper-arid deserts (e.g., Pachur and Altmann 2006), the perception of environmental changes and especially human impact in Central Asian deserts is controversial.

This is particularly the case when considering forest distribution during the mid-Holocene climatic optimum and the Subboreal forest decline. In contrast to fragmentary evidence for dark taiga replaced by steppe following fire in the Gobi Altay after 4,000 yr BP (Dinesmann 1989, cited in Gunin et al. 1999) and the far reaching conclusions possible from forest plant disjunctions (Jäger 2005), palaeoclimatic maps of Frenzel et al. (1992) and Petit-Maire and Bouysse (2002) for Central Asia show steppe for the mid-Holocene climatic optimum. This observation is however not consistent with higher humidity during the Atlanticum which is confirmed with regional deviations by multi proxies of northern China (An et al. 2006). It is rarely even discussed whether the recent treelessness of Central Asia may have been, at least partially, influenced by humans. While evidence exists for fires ignited by Palaeolithic steppe hunters in the forest steppe ecotone of southern Siberia (Blyakharchuk et al. 2004), evidence connecting humans to forest fires in the Subboreal forest refugium of southern Mongolia has been reported (Miehe et al. Ms.in Review). For the Tibetan Plateau, the evidence is less divergent. During the mid-Holocene climatic optimum, ‘‘temperate deciduous forest’’ is mapped in arid Southern Tibet westwards to the Indus Yarlung Zhangbo watershed (Petit-Maire and Bouysse 2002; Tang and Shen 1996). *Pinus* and *Quercus* forest (Yu 2004) is even claimed in the Central Tibetan Highlands with alpine steppe and discontinuous permafrost in altitudes of 4,500 m (Atlas of Tibet 1990). The possibility of long distance dispersal of pollen is however not considered. Van Campo et al. (1996) could even demonstrate modern pollen rain of *Pinus*, *Tsuga* and *Quercus* in the alpine deserts of western Tibet at 5,000 m elevation and with 50 mm/a precipitation.

A highly confusing contradiction concerns the ‘‘alpine’’ pastures of north-eastern Tibet with forest relicts (Zhang et al. 2005) in a forest climate with 500–600 mm/a precipitation and summer temperatures well above 10°C (Miehe et al. 2001). *Picea* and *Juniperus* forest had been detected there for the mid-Holocene optimum but their decline has been explained by the adverse Subboreal climatic conditions (Herzschuh et al. 2006; Da et al. 1989). Even the review of Ren (2000) underestimates the capacity of even small numbers of humans to clear forests for rangeland using fire as a tool. He concedes human interference on the Tibetan Plateau only in the last millennium and explains this late impact by the remoteness of the area—which is certainly true from the standpoint of a sedentary agricultural society with high population density in the Chinese mainlands. He accepts human interference in the forest belt of the eastern declivity of the Tibetan Plateau since 2,000 yr BP whereas Thelaus (1992) and Frenzel (1994) see human impact on forest since 5,000 yr BP.

The presence of humans in Southern Tibet is documented by hand and footprints of hot springs for the Last Glacial Maximum (Zhang and Li 2002), through upper Palaeolithic sites, and a number of Neolithic sites (Chayet 1994; Fu et al. 2000; Aldenderfer and Zhang 2004). Currently only a single pollen diagram from arid Southern Tibet shows human impact. The ‘‘Lhasa 1’’ site (3648 m, 29°10′ N/91°04′ E) shows the decline of *Juniperus* forests since 4,600 yr BP with the presence of pollen indicative of human use and cereal pollen in parallel with *Juniperus* charcoal remains of the same period, and intensified erosion (Schlütz 1999; Kaiser et al. 2006; Miehe et al. 2006). The authors suggest that there were *Juniperus* forests in Southern Tibet, which had been cleared by humans. This supports Zhang (1988: 40) who lists *Juniperus tibetica* stands in the Yarlung Zhangbo gorge east of Xigaze, Namling, Damxung, Reting and Nagarze (appendix: 14, 15, 31, 33, 48). Zhang assumes already that human impact destroyed the forests. In contrast Lauer et al. (1996) interpret the current desert-like vegetation as natural. Similarly, Song et al. (2004) state that the vegetation of Tibet is undisturbed. *Juniperus* is not considered in their models of tree species distribution. Thus, it is evident on the one hand that the current treelessness of arid environments of Southern Tibet is considered by some authors to be natural. On the other hand, other authors suggest that the current degraded pastures of Southern Tibet are human-caused. The results of a forest inventory in Southern Tibet since 1984 (Fig. 1) may contribute to the elucidation of the human dimension of global change. The hypothesis of the present paper is that isolated tree stands in the arid environments of Southern Tibet are relicts of a once closed forest belt, which had been fragmented. The absence of forests in non-water-surplus habitats today is considered to be human-caused. Although the region's population density is relatively low, people were able to replace forests with pastures and create the present cultural landscape of semi-desert rangelands.

Study area

Location

The area under consideration belongs to the Yarlung Zhangbo catchment of Southern Tibet (Xizang Autonomous Region, China) in the rain shadow of the Himalayas. The locations where forest relicts were recorded stretch from the current western border of the mountain forests of the south-eastern Himalayas (approx. 93°E) 650 km to the west (approx. 86°E) roughly between 28°30′ N and 30°30′ N. The valley bottoms rise from 3,200 in the east to 4,200 m in the west. Slopes mostly are smooth and easily accessible with a few intermittent gorges with precipices providing safe sites for trees.

Climate

Southern Tibet has a pronounced highland climate of the subtropics. The radiation values regularly exceed the solar constant especially during fair weather conditions with cumulus clouds in summer (Kuhle and Jacobsen 1988). Evaporation is especially high in the main valleys with the reflection of the cloud bands of the valley air circulation system and the daily valley winds. Penman-Monteith potential evaporation estimates of Lhasa amount to 1,328 mm/a and in Xigaze to 1,259 mm/a (Thomas and Chen 2002). The Transeau ratio of Lhasa is 0.379 and of Xigaze 0.217 (Böhner and Lehmkuhl 2005). This would support Henning's (1994) conclusion that the threshold of forests equates a Transeau ratio of 0.2. The fact that trees are found in extremely wind-exposed arid run-off-sites in the vicinity of Xigaze (appendix: 14) supports the validity of the Transeau ratio. The

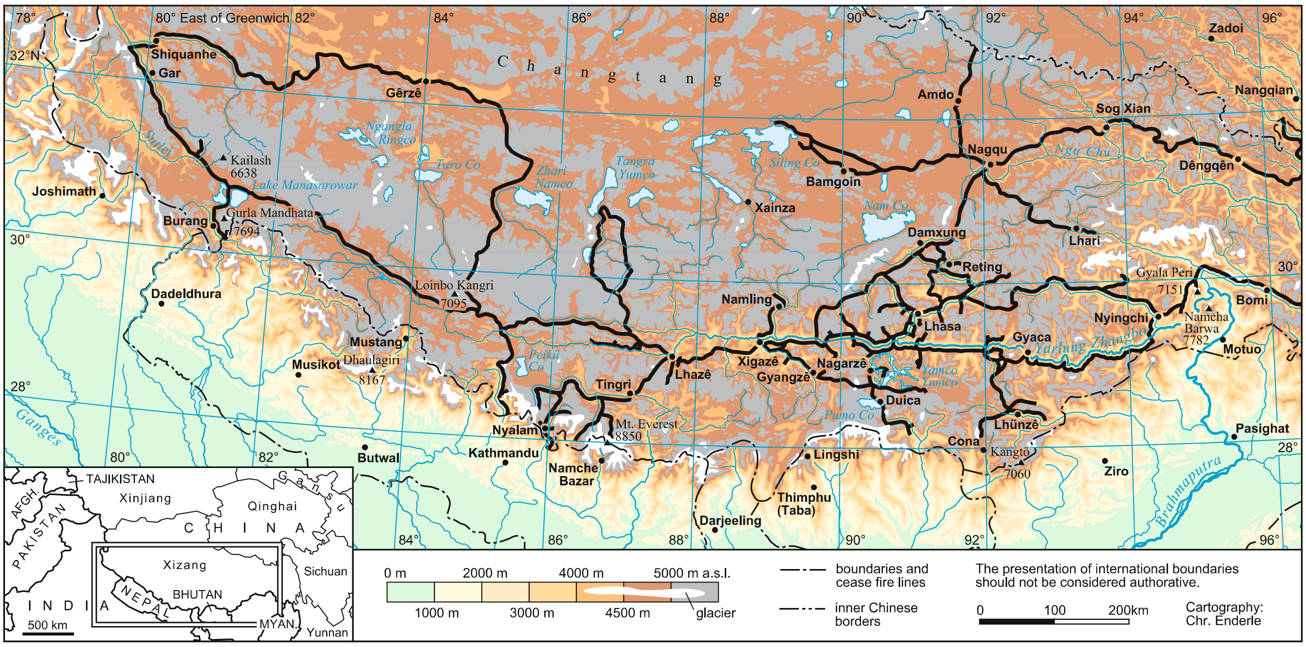


Fig. 1 Survey routes of the inventory, 1984, 1993–1995, 1997–1999, 2001–2006. Base map altered from Miehe et al. (2001)

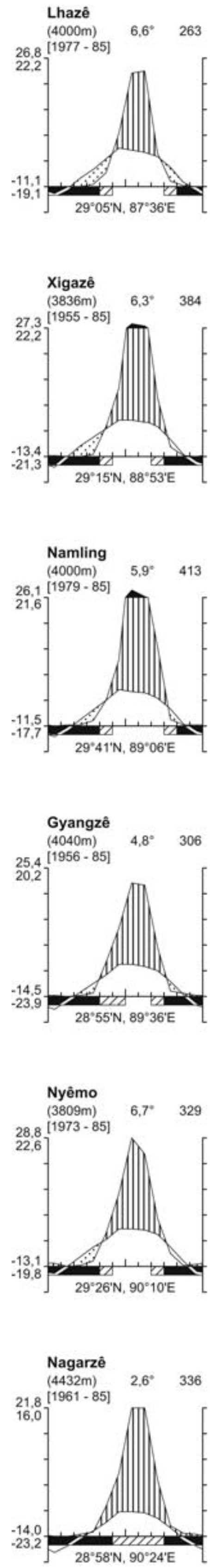
conclusion however that a given evaporation value inhibits tree growth is defeated with the factual presence of vigorous trees.

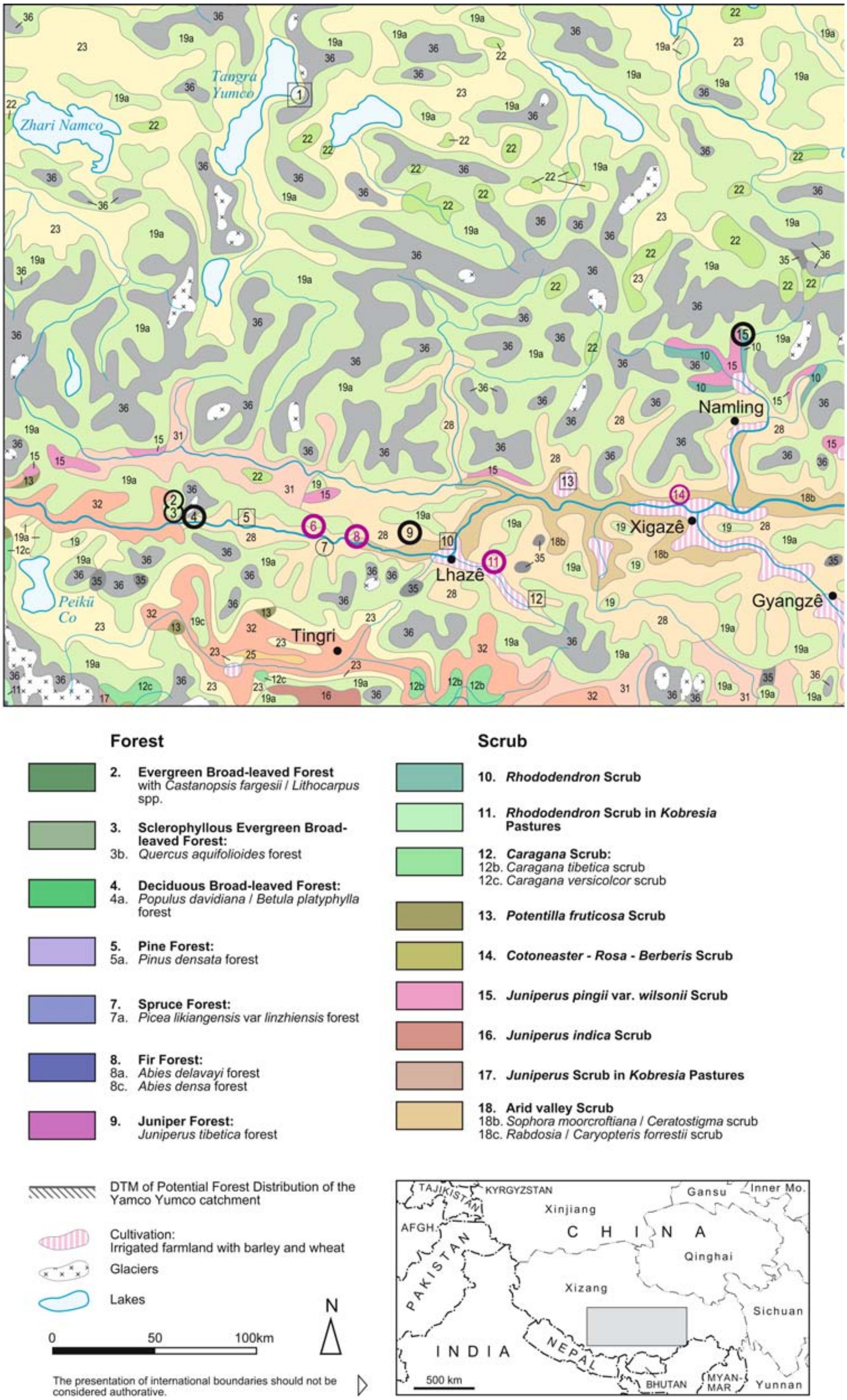
The rainy season starts in mid-June and lasts until mid-September; torrential rainfall is common, sometimes with hail in the late afternoon or during night time after thunderstorms. Advective rainfall lasting for a few days is usual during the end of the rainy season in September bringing the first snow in alpine areas above 4,800 m. Snowfall in winter is rare and the snow melts within hours at least on the sunny slopes. Especially for *Juniperus*, which clearly prefer southern exposures, snow is certainly not a decisive ecological factor. Rainfall data provided by the Meteorological Service of China give the minimum possible values, because all climate stations are situated in the centre of cloudless arid valley bottoms with strong daily up-valley winds. However, even the data of the most arid sites indicate enough rainfall to allow for forest: along the Yarlung Zhangbo the rainfall decreases from approx. 400 mm/a to 260 mm/a westwards (see Fig. 2). Locations in the vicinity of higher mountain massifs attain higher rainfall and this is applicable as well to the upper side valleys. Rain-gauge measurements on southern slopes above Lhasa since 1997 reveal values reaching nearly twice the rainfall of the valley bottom in altitudes of the upper treeline ecotone in 4,650 m, 1,000 m higher than Lhasa (Miehe et al. 2004). Despite the high altitudes temperatures are suitable for forests: between May and September mean monthly temperatures are above 10°C. The growing season with mean monthly temperatures above 5°C covers 8 months. On the southern exposures where *Juniperus* usually grow, temperatures are certainly even higher than the data delivered from the climatic stations. Soil temperatures in altitudes of the upper treeline ecotone in 10 cm soil depth of southerly exposures show daily ranges of 15 K during fair weather. Maximum subsoil temperature reached 15–20°C during winter. Only occasionally did subsoil temperatures drop below 0°C. Thus permanently frozen ground during winter in fair weather as a precondition for frost drought is not probable. Ice and frost heave of open soil, which may destroy seedlings, occurs rarely because the soil is mostly too dry to allow the formation of needle ice. Thus the climate of Southern Tibet between 86°E and 93°E can be classified as a forest climate. This contrasts to the treeless, arid appearance of the pastures of Southern Tibet.

Fig. 2 The vegetation of Xizang (changed from Zhang 1988),▶ with climatic diagrams (based on data of the Meteorological Service of China) and *Juniperus* tree sites (see appendix)

Vegetation and human impact

The vegetation of Southern Tibet reflects the effects of desertification through fuel wood extraction of all woody plants mostly with the roots (including the most spiny dwarf shrubs), the effects of soil compaction and trampling of livestock leading to gully erosion of the loamy, deeply weathered bedrock and the long lasting result of selective grazing leaving only plants of little grazing value with rhizomes, bulbs, spines or repellent characters of the leaves. The irregularly rhombic pattern of cattle tracks on the slopes displays the most important character of the vegetation structure. The vegetation structure is diffuse but patchy and the presence of plants useful for fuel or grazing exhibit clear gradients from the irrigation oasis. All shrubs, herbs and graminoids suitable for fuel or grazing are missing around the settlements and only in greater distance to the villages does the vegetation cover increase and the shrubs may attain their natural habit. The most common phanerophytes in Southern Tibet (*Sophora moorcroftiana* (Benth.) Benth. ex Baker, *Artemisia santolinifolia* Turcz. ex Bess., *Buddleja crispa* Benth., *Cotoneaster* spp.) display a dwarf habit of rarely more than half a metre, but grow as shrubs of 2 m or even as multi-stemmed trees of 5 m height in safe sites. Grasses grow mostly in the protection of spiny shrubs; only *Pennisetum flaccidum* Griseb. is common below 4,000 m and is not preferably grazed. *Carex duriuscula* is as common and widely grazing-resistant. Amongst the herbs Lamiaceae, Boraginaceae and aromatic Asteraceae or those with woolly leaves (*Artemisia* spp., *Anaphalis* spp., *Leontopodium* spp., *Pulicaria insignis* Drumm. ex Dunn, *Dolomiaea* spp.) prevail. *Stellera chamaejasme* L., *Arisaema flavum* (Forssk.) Schott or *Iris* spp. are common grazing weeds of more humid pastures. Where slopes are not permanently affected by hoofs and claws


Lhazê
(4000m) 6,6° 263
[1977 - 85]
26,8
22,2
-11,1
-19,1
29°05'N, 87°36'E
Xigazê
(3836m) 6,3° 384
[1955 - 85]
27,3
22,2
-13,4
-21,3
29°15'N, 88°53'E
Namling
(4000m) 5,9° 413
[1979 - 85]
26,1
21,6
-11,5
-17,7
29°41'N, 89°06'E
Gyangzê
(4040m) 4,8° 306
[1956 - 85]
25,4
20,2
-14,5
-23,9
28°55'N, 89°36'E
Nyêmo
(3809m) 6,7° 329
[1973 - 85]
28,8
22,6
-13,1
-19,8
29°26'N, 90°10'E
Nagarzê
(4432m) 2,6° 336
[1961 - 85]
21,8
16,0
-14,0
-23,2
28°58'N, 90°24'E


Tangra Yumco
Zhari Namco
Peikü Co
Tingri
Lhazê
Xigazê
Namling
Gyangzê
Forest
2. Evergreen Broad-leaved Forest with Castanopsis fargesii / Lithocarpus spp.
3. Sclerophyllous Evergreen Broad-leaved Forest:
3b. Quercus aquifolioides forest
4. Deciduous Broad-leaved Forest:
4a. Populus davidiana / Betula platyphylla forest
5. Pine Forest:
5a. Pinus densata forest
7. Spruce Forest:
7a. Picea likiangensis var linzhiensis forest
8. Fir Forest:
8a. Abies delavayi forest
8c. Abies densa forest
9. Juniper Forest:
Juniperus tibetica forest
Scrub
10. Rhododendron Scrub
11. Rhododendron Scrub in Kobresia Pastures
12. Caragana Scrub:
12b. Caragana tibetica scrub
12c. Caragana versicolcor scrub
13. Potentilla fruticosa Scrub
14. Cotoneaster - Rosa - Berberis Scrub
15. Juniperus pingii var. wilsonii Scrub
16. Juniperus indica Scrub
17. Juniperus Scrub in Kobresia Pastures
18. Arid valley Scrub
18b. Sophora moorcroftiana / Ceratostigma scrub
18c. Rabdosia / Caryopteris forrestii scrub
DTM of Potential Forest Distribution of the Yamco Yumco catchment
Cultivation: Irrigated farmland with barley and wheat
Glaciers
Lakes
0 50 100km
N
The presentation of international boundaries should not be considered authorative.
TAJIKISTAN
KYRGYZSTAN
Xinjiang
Gansu
Inner Mo.
AFGH.
PAKISTAN
C H I N A
Qinghai
Xizang
Sichuan
INDIA
NEPAL
BHUTAN
MYAN-MAR
Yunnan
500 km

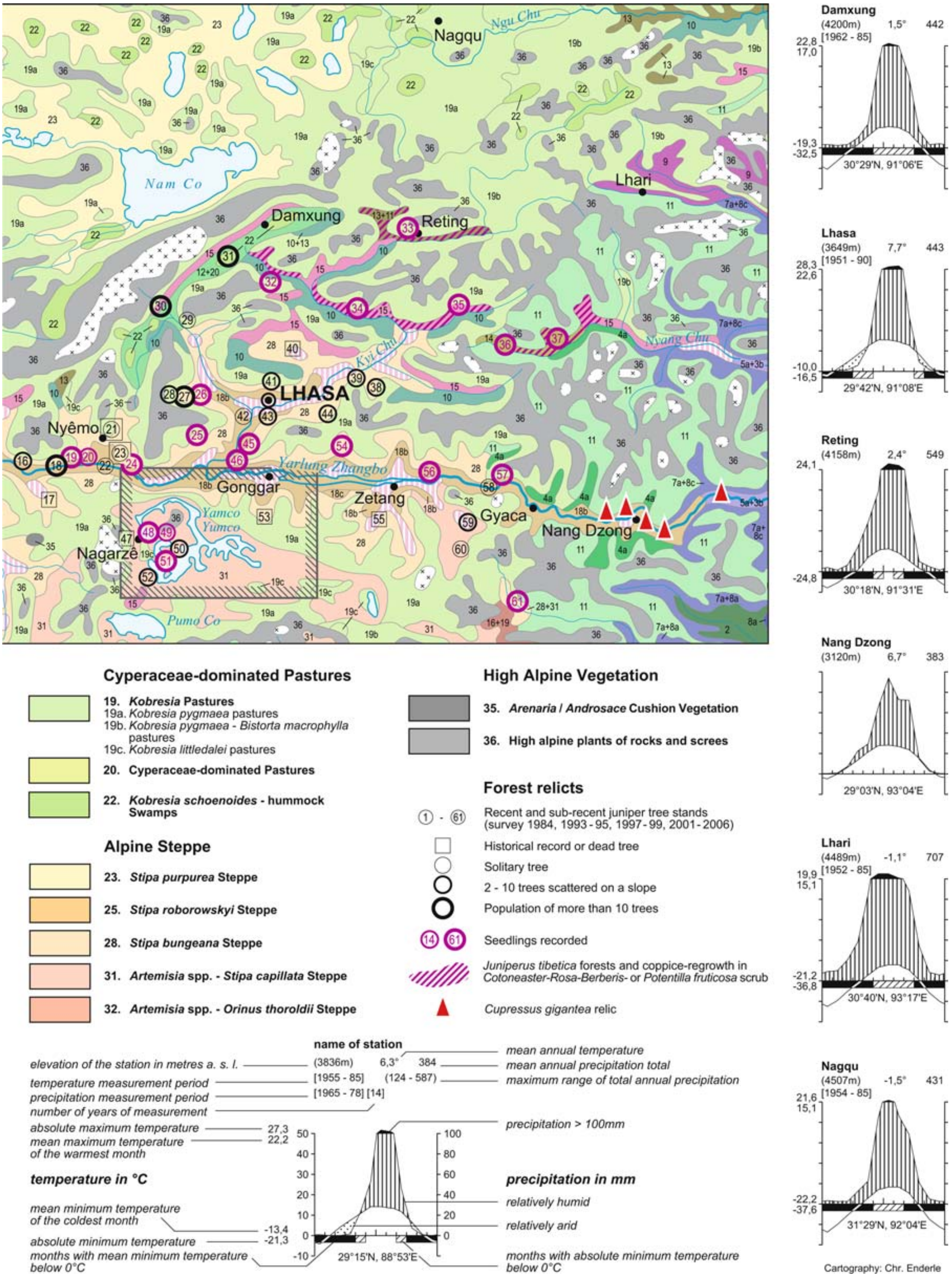


Fig. 2 continued

surfaces are sealed by Hepaticae *(Riccia)* or Pottiaceae and Cyanophyceae or the rosettes and carpets of *Selaginella* spp.

The ''Vegetation of Xizang'' (Zhang 1988) describes a larger number of vegetation units named after dominant species (see Fig. 2). The vegetation units roughly described here are mostly found on southerly exposures. Despite the existence of a few records of *Juniperus* on north-facing cliffs surrounded by dwarf thickets of rhododendrons, *Juniperus* of Tibet are primarily bound to sunny slopes.

Most of the relictual tree stands of our inventory are located in open dwarf shrublands of *Sophora moorcroftiana*. These heathlands dominate the river terraces and the colluvial soils of the lower slopes. They are regularly cut for tinder and resprout from the base. These shrublands are in the daily range of goats and sheep using the nearest pastures around the settlements. Annual grazing weeds (Chenopodiaceae, Polygonaceae, Boraginaceae) are common and the total plant cover rarely exceeds 50%. As soon as grazing is excluded, recovery takes place, starting with grasses; within a few years the plant cover reaches 100% (Miehe et al. 2004).

The second largest pasture type is widespread in intramontane basins of Southern Tibet mostly in altitudes above 4,000 m. Various *Artemisia* spp. are dominant, first of all *Artemisia santolinifolia*; in more strongly degraded sites annual *Artemisia* spp. prevail. The most common grasses are *Stipa* spp. Even in altitudes well below the upper treeline cushion plants of alpine origin like *Androsace tapete* Maxim. and *Arenaria kansuensis* Maxim. occur. Open dwarf shrublands of *Potentilla fruticosa* L., *Berberis* spp., *Rosa* spp. and *Cotoneaster* spp. are mostly found on slopes beyond the radius of easy fuel wood gathering or around monasteries where fuel wood eradication is sometimes forbidden. They are mostly confined to steep, rocky slopes, likely owing to the more difficult access. *Potentilla fruticosa* however colonizes open soil patches as does *Juniperus*. The flora of these shrubberies is nearest to that of *Juniperus* forest relicts and they probably represent an early degradation type. This is as well the case where only shrubby *Juniperus pingii* W.C. Cheng ex Ferré v. *wilsonii* (Rehder) Silba are left and the *Juniperus* trees had been cut and removed. These are the only current remains of larger *Juniperus* resources in Southern Tibet. They are rapidly vanishing. Where they are accessible along roads they have been eradicated, carried away with vans to Lhasa and sold at night.

The *Kobresia* pastures of the study area are the southwestern extension of the largest pasture type of the southeastern half of the Tibetan Plateau. They are still believed to be an alpine vegetation formation (Zhang 1988; Yu et al. 2001; Song et al. 2004). Their successional status is unclear because open soil patches surrounded by the felty turf of *Kobresia pygmaea* (C.B. Clarke) C.B. Clarke are colonized by *Juniperus* groves up to 4650 m. However, *Juniperus* never establishes directly in the *Kobresia pygmaea* pastures but only where the sods have been removed through chafing of yaks, soil-dwelling pika or marmots, rockfall, slope movements or gelifluction (Miehe et al. 1998).

Summing up, there is a wide range of pasture types in Southern Tibet over 1,600 m in altitude where *Juniperus* trees can be found.

Unlike *Juniperus communis* L. in European heathlands, the *Juniperus* of Tibet do not profit from uncontrolled grazing even they are not intentionally browsed. The worst grazing impact affects the seedlings by trampling and grazing of yaks, which do not graze selectively as do sheep or goats. As the southern exposures in altitudes below 4,800 m are the best winter pastures and the nearest to settlements, the grazing impact is strongest during winter.

Material and methods

Plant names are given according to ''Flora Xizangica'' (Wu 1983–1987); the taxonomy of Cupressaceae follows Farjon (2005).

Vegetation surveys undertaken in Southern Tibet since 1984 (Fig. 1) provided information about isolated *Juniperus* stands or potential *Juniperus* forest islands in currently treeless environments. The genus *Juniperus* includes mostly trees and a few caespitose phanerophytes such as *Juniperus communis* or *Juniperus sabina* L. The *Juniperus* of High Asia however show a very flexible growth habit and can grow as a shrub under adverse conditions or after disturbances (e.g., fire or logging). Thus along an altitudinal gradient there is a decline in height from several metres to a half a metre and a transition from a single-stemmed tree to a multi-stemmed tree merging into a globular bush of 2 m or less until the size of a dwarf shrub (0.5 m) is attained. These transitions are not unique but typical treelines (e.g., Miehe and Miehe 2000a) and therefore it is adequate to refer to ecotones rather than to distinct borderlines. In Tibet woodlands of globular bushes are widespread where tree stands had been destroyed by logging during the Cultural Revolution and now exhibit a coppice regrowth, which will lead to a forest of multi-stemmed trees. As timberline descriptions are often difficult to compare due to lack of precise information about size and growth form, the list in the appendix gives the height of the *Juniperus*, regrowth form and the diametre at breast height. In the strict sense a tree is a phanerophyte with a stem and a crown regardless the size, but it is admitted that trees shorter than one metre are very rare. As the life form is not defined by a quantity (e.g., ‘‘smaller than 2 m: bush; larger than 2 m: tree’’) it is not meaningful to communicate imprecise measures. To avoid misunderstandings the appendix gives life form and the height. The diversity of classification concepts of vegetation formations makes it necessary to premise that a forest consists of trees, although the anglophone world knows ‘‘woodlands’’ as a second tree formation. Most tree stands of Southern Tibet would refer to ‘‘woodlands’’. To avoid misunderstandings we only use the term ‘‘forests’’ because the present status of relictual trees makes it difficult to decide which crown cover degrees a recovered tree community would achieve.

Juniperus are sacred trees according to the beliefs of the local Buddhist population; the trees are worshipped religiously and are part of the national identity of Tibetans. Accordingly there is a common knowledge about tree stands and their fate in the past. Interviews were held with the help of English-speaking Tibetans. The interviews were semi-formal in the sense that they were opened with the specific questions about what the local informant knows about *Juniperus* in the area. In many cases the place of the (rebuilt) Buddhist temple (‘‘gompa’’) was the best spot to gain information about recent to sub-recent tree stands because most gompas had a sacred forest. The Tibetan language differentiates between *Juniperus* trees and coppice regrowth forming mostly a globular bush of one to two metres on one hand and dwarf-shrub *Juniperus* on the other hand. This is useful for identification of species: As there are only two *Juniperus* tree species west of 93°E (*Juniperus tibetica* and *Juniperus convallium*) information about sites must refer to one of these species, whereas information about dwarf shrubs refer to *Juniperus pingii* var. *wilsonii*.

In addition, written sources were evaluated. This includes travel journals of foreigners reporting about their travels in Tibet (e.g., the Japanese Buddhist monk Katoxitu in the 1920s; Peter Aufschnaiter in the late 1940s). Both sources were cross-checked by local informants. Information of the pundit Sarat Chandra Das (1902) could not be confirmed and was omitted. Information in Tibetan religious or historical literature was another valuable incidental source. Most of the texts had not been edited but relevant information was cited here as personal communication of tibetologists (e.g., Per Sörensen, University of Leipzig). So far, early evidence of *Juniperus* forests as derived from historical documents had been crossed-checked with the help of pollen analysis only in Lhasa (Schlütz in Miehe et al. 2006). In a few of the sites cited in Fig. 2 tree ring analysis had been carried out giving some information about the age of the oldest trees and climatic shifts (Bräuning 1999). Most of the recent sites had been checked in the course of vegetation records. Recorded parameters included GPS location, altitude (Thommen altimeter and/or maps), growth and structure (if forests), seed production, distribution of monoecy or dioecy, presence of seedlings or younger trees, drought damage, parasites (*Arceuthobium oxycedri* (DC.) M.Bieb.), grazing impact, and human interference (incense, fuel wood or timber extraction). In selected sites used as gene pools for nursery purposes, seeds where checked for vitality (Miehe et al. 2003). In 14 sites seedling data could not be obtained due to inaccessibility of the locality (e.g., rock walls, single trees in yards, remote slopes); where records rely on local informants only data about rejuvenation were not available (two sites).

The potential natural distribution of *Juniperus* (Fig. 6) was mapped using GRASS (Geographical Resources Analysis Support System) version 5.0.

Results and discussion

Inventories in the pastures of Southern Tibet since 1984 revealed more than 60 isolated sites of *Juniperus* trees in an environment, which has in the common consensus of the scientific community no potential for tree growth except on sites with water surplus such as floodplains with natural *Hippophae*- or *Salix* thickets. The inventory includes 13 locations where *Juniperus* trees or forests were known in the past from historical documents or oral tradition, but destroyed during warfare or during the Cultural Revolution. Out of the 51 locations of present-day tree stands 24 sites are forests covering an area of more than one square kilometre. Four sites cover an area between one and five ha. Around 14 sites have between two and ten trees; seven sites consist of only a single tree. The western-most trees of *Juniperus tibetica* to date were found 650 km west of the current forest border near Nang Dzong. The fact that *Juniperus* trees are adored as sacred trees in the believes of Buddhist Tibetans means that the local population knows every stand in the region as a religious landmark. Interviews revealed that these trees mapped in Fig. 2 (appendix: 2) are known to be the western-most in Southern Tibet. The altitude of these trees is 4850 m. They represent the highest tree stands of the northern hemisphere (Miehe et al., in review MRD). *Juniperus* in similar altitudes are rare and mostly only globular shrubs of half a metre can be found. In the Yamco Yumco basin all transect studies revealed a greater number of small individuals towards higher altitudes in parallel with linear decrease of growth height (see Fig. 5). It is uncertain if this reflects the impact of adverse growing conditions or an upslope shift of the forest line due to global warming. Tree ring analysis (Bräuning 1999) and evaluation of climatic data (Böhner 1996) however show a recent rise of temperatures in the area,although between 1961 and 2000 the potential evatranspiration has decreased (Chen et al. 2006). Yet it has to be considered that the treeline ecotone is less affected by grazing and trampling than the foot of the slopes, which are closer to settlements.

The northern-most tree records towards the alpine steppe of the Central Tibetan Highlands (''Changtang'') are situated on south-facing slopes of valleys bordering the Changtang (Fig. 2: 1, 15, 30–33) and on the eastern bank of the Tangra Yumco. So far there have been no records of trees or macrofossil remains such as determined charcoal in the Changtang.

All of the *Juniperus* species of High Asia are restricted to open sandy or rocky soils. They do not grow in humic, densely rooted soils of grasslands or Cyperaceae mats; sites with standing water are also avoided. They are found on all kinds of weathered bedrock, in limestone cliffs as well as between granite boulders or in slate screes. All records (except no. 42 and 58) cited in the appendix are not restricted to sites with a water surplus, nor do they exhibit a special preference for any favourable microclimatic conditions. Field evidence suggests that most tree stands occupy normal sites. Nursery experiments in Lhasa revealed that *Juniperus* develop very deep roots (1 year after germination seedlings of 3–5 cm height had tap-roots of 30–40 cm) and it can be assumed that old trees survive drought through their deep reaching roots. Thus the presence of native trees even in most exposed sites (Fig. 4) does not necessarily imply that young trees can establish today. Records of seedlings therefore are a pre-requisite for the conclusion that the juniper populations are vigorous and that the area where isolated trees had been recorded is potentially forested.

Out of the 51 present tree stands no seedling records have been obtained in 23 sites. From these 14 sites were not accessible (trees on remote slopes, in steep rock walls or yards); from two sites the local informants did not provide data about seedlings; in six sites no seedling data were recorded.

For the remaining 28 sites records on rejuvenation exist. In 23 out of these 28 sites seedlings could be traced. The majority of populations showing rejuvenation (20 out of 23—see Table 1) belong to size class 3 thus being made up of at least 10 trees. This is not surprising as it has been observed frequently that the germination rate of individual trees vary significantly thus the probability of a mother tree increases with population size apart from apparent effects of genetic drift in small populations.

It is striking that from the 25 populations with more then 10 trees for which regeneration records exist only the two northernmost populations (15, 31) bordering the Central Tibetan Highland of the Changtang do not show any signs of rejuvenation. Here it can be assumed that the conditions of establishment of young trees are unfavourable in the moment due to climatic reasons and due to strong grazing pressure.

The other three cases where a total absence of current rejuvenation is confirmed are solitary trees extremely exposed to trampling and grazing. In the

Table 1 Rejuvenation in present juniper stands of Southern Tibet

No[a]	Location	Elevation	Species	Size class[b]	Rejuvenation
Populations with rejuvenation					*23*
8	29°09′ N/87°09′ E,	4,250–4,650 m	*J. tibetica*	3	Seedlings
11	29°06′ N/87°57′ E	4,300–4,750 m	*J. tibetica*	3	Seedlings
14	28°21′ N/88°42′ E	3,980 m	*J. convallium*	2	Seedlings
19	29°20′ N/89°57′ E	3,980–4,850	*J. convallium, J. tibetica*	3	Seedlings
20	29°22′ N/90°09′ E	4,080 m	*J. convallium*	2	Seedlings
24	29°20′ N/90°21′ E	4,350–4,500 m	*J. tibetica*	3	Seedlings
25	29°28′ N/90°43′ E	4,100–4,300 m	*J. tibetica*	3	Seedlings
26	29°45′ N/90°41′ E	4,340–4,450 m	*J. tibetica*	3	Seedlings
32	30°15′ N/91°17′ E	4,100 m	*J. tibetica*	3	Seedlings
33	30°18′ N/91°31′ E	4,200–4,860 m	*J. tibetica*	3	Seedlings
34	30°05′ N/91°33′ E	4,000–4,800 m	*J. tibetica*	3	Seedlings
35	30°00′ N/92°02′ E	4,300–4,600 m	*J. tibetica*	3	Seedlings
36	29°54′ N/92°27′ E	4,470 m	*J. tibetica*	3	Seedlings
37	29°57′ N/92°51′ E	4,600 m	*J. tibetica*	3	Seedlings
45	29°27′ N/91°01′ E	4,300–4,480 m	*J. tibetica*	3	Seedlings
46	29°22′ N/90°53′ E	3,600–4,200 m	*J. convallium*	3	Seedlings
48	28°59′ N/90°26′ E	4,400–4,650 m	*J. tibetica*	3	Seedlings
49	28°59′ N/90°28′ E	4,450–4,550 m	*J. tibetica*	2	Seedlings
51	28°47′ N/90°30′ E	4,450–4,850 m	*J. tibetica*	3	Seedlings
54	29°22′ N/91°32′ E	4,070–4,600 m	*J. tibetica*	3	Seedlings
56	29°16′ N/91°57′ E	3,650–4,600 m	*J. convallium, J. tibetica*	3	Seedlings
57	29°18′ N/92°08′ E	3,800–4,770 m	*J. tibetica*	3	Seedlings
61	28°36′ N/92°32′ E	4,070 m	*J. tibetica*	3	Seedlings
Populations without rejuvenation					*5*
15	30°03′ N/89°06′ E	4,410–4,470 m	*J. tibetica*	3	No seedlings
31	30°23′ N/90°54′ E	4,250–4,300 m	*J. tibetica*	3	No seedlings
42	29°38′ N/91°00′ E	3,640 m	*J. tibetica*	1	No seedlings
58	29°14′ N/92°00′ E	3,560 m	*J. convallium*	1	No seedlings
60	29°02′ N/92°12′ E	4,150 m	*J. tibetica*	1	No seedlings
Populations with no rejuvenation data[c]					*23*
1	31°06′ N/86°48′ E	4,590 m	*J.* spec.	1	No data
2	29°14′ N/86°15′ E	4,850 m	*J. tibetica*	2	No data
3	29°12′ N/86°16′ E	4,850 m	*J. tibetica*	2	No data
4	29°93′ N/86°18′ E	4,200–4,600	*J. tibetica*	3	No data
7	29°09′ N/86°55′ E	4,350	*J. tibetica*	1	No data
9	29°19′ N/87°23′ E	4,440–4,600 m	*J. tibetica*	3	No data
16	29°18′ N/89°44′ E	4,200 m	*J.* spec.	2	No data
18	29°19′ N/89°56′ E	3,820–4,000 m	*J. convallium*	3	No data
21	29°24′ N/90°10′ E	3,850 m	*J. convallium*	1	No data
22	29°20′ N/94°14′ E	3,720 m	*J. convallium*	1	No data
23	29°22′ N/90°20′ E	4,080 m	*J. tibetica*	1	No data
27	29°44′ N/90°42′ E	4,050–4,400 m	*J. tibetica*	3	No data
28	29°44′ N/90°44′ E	4,500–4,700 m	*J. tibetica*	2	No data
29	30°03′ N/90°35′ E	4,300 m	*J. tibetica*	1	No data

Table 1 continued

No[a]	Location	Elevation	Species	Size class[b]	Rejuvenation
30	29°50′ N/90°21′ E	4,300 m	*J.* spec.	3	No data
38	29°42′ N/91°40′ E	4,200 m	*J. tibetica*	2	No data
39	29°53′ N/92°20′ E	4,050 m	*J.* spec.	2	No data
41	29°43′ N/91°07′ E	4,600 m	*J. tibetica, J. convallium*	2	No data
43	29°35′ N/91°07′ E	3,680 m	*J. convallium*	2	No data
44	29°33′ N/91°24′ E	4,200 m	*J. tibetica*	2	No data
50	28°58′ N/90°35′ E	4,500–4,600 m	*J. tibetica*	2	No data
52	28°52′ N/90°22′ E	4,400–4,600 m	*J. tibetica*	2	No data
59	29°04′ N/92°10′ E	3,0900 m	*J. tibetica*	2	No data
Population total					*51*

[a] No. refers to the number of the population description in the Appendix. They are grouped geographically—No.1 being the westernmost population and No.61 the easternmost

[b] Size Classes used: class 1 refers to a solitary tree, class 2 refers to 2–10 trees scattered on a slope, class 3 refers to a population of more then 10 trees

[c] Population with no rejuvenation data: the sites were not accessible or data could not be obtained

case of site 42 we may additionally assume that the female tree is too old to produce fertile seeds.

The 23 populations with rejuvenation are distributed almost over the entire remaining range regardless of the longitude and thus despite the westwards decreasing rainfall.

However young trees (approx. 5–20 years) are extremely rare and most stands seemed overaged. Some populations show great changes in fructification others are constantly extremely rich. The discrepancy of luxuriantly fruiting trees and the scarceness of seedlings is striking. This phenomenon has been observed in other juniper species worldwide but is not yet well understood (e.g., *Juniperus communis* in Germany, Michalczyk in prep.; *Juniperus sabina* in the Gobi Altay of Southern Mongolia, Wesche et al. 2005). Both, the fertility of the embryos as investigated through tetrazolium tests (less then 3% of the embryos investigated in the Reting Forest were fertile, Opgenoorth unpublished data) as well as germination rates are generally low, especially in the largest and overaged forests (seeds from Reting have germination rates of 1%, Meng unpublished data). Even after exclosure of livestock in Reting Forest only very few mother trees had seedlings and only few seedlings survive the first winter. As dead seedlings could not be found, it is assumed that hares, pika, mice and scratching birds extinguish the seedlings. In all other tree stands seedlings only were detected in safe sites (e.g., between boulders or at cliffs, in the shelter of thorny shrubs). As *Juniperus* is bound to southern exposures and those sunny slopes are the winter pastures, grazing pressure is extreme leaving only safe sites for rejuvenation. Thus the effect of low germination rate of overaged trees and the scarceness of safe sites lead to an extremely poor regeneration. As the junipers of Tibet reach an age of 800 and more years (Bräuning 1999) very few establishing events are needed for the survival of the forests.

In addition, tree ring analysis even of the drier sites (appendix: 48) shows no specific borderline symptoms (Bräuning 1999). The final proof that isolated tree stands show a forest potential is provided by a successful reforestation trial with *Juniperus convallium, Juniperus tibetica* and *Cupressus gigantea* W.C. Cheng & L.K. Fu on exclosure plots of southern slopes above Lhasa where *Juniperus* and *Cupressus* have grown since 1999 without irrigation (Miehe et al. 2003).

Most *Juniperus* relicts cited in our inventory are located on southern exposures. There are however exceptions which permit one to draw various scenarios of the potential altitudinal forest belts in Southern Tibet, as given in Fig. 3. Furthermore, by comparing all *Juniperus* sites in Southern Tibet along a humidity

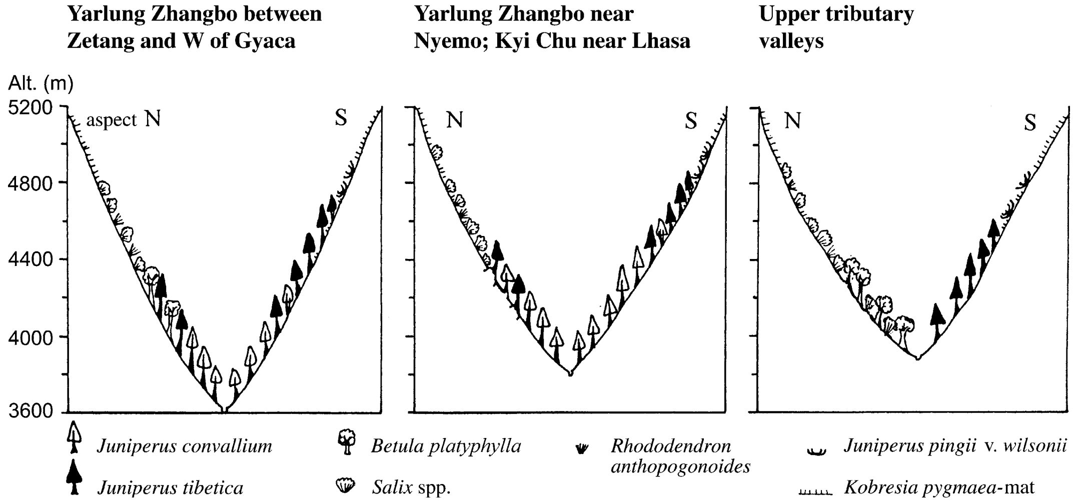


Fig. 3 Natural altitudinal forest belt types of Southern Tibet derived from isolated tree stands and vegetation records. The sketch only shows the dominant species. Draft S Miehe et al. (2002)

gradient starting in the southwestern-most arid locations (appendix: 6, 7, 50, 51) towards the humid *Picea*- and *Abies*-forests of southeastern Tibet we are able to apply principal ecological laws of plant ecology: In the arid locations *Juniperus* grow on all exposures including open slopes of strictly northern exposure. Along with increasing humidity the number of phanerophytic species increases and *Juniperus* are only found on sunny exposures, leaving shady slopes to *Betula* forests with *Rhododendron* thickets in the understorey or at the treeline ecotone. In such areas, *Juniperus* are only found on exposed cliffs (appendix: 37). Farther to the southeast, where spruce and fir forests cover all slopes, *Juniperus* are rare and restricted to south-facing cliffs. The gradual change of biotope is consistent with the ''ecological law of relative habitat constancy and changing biotope'' (Walter and Walter 1953; Miehe 1986).

The two *Juniperus* tree species of Southern Tibet show a distinct distribution. *Juniperus convallium*, which was so far not known from Southern Tibet (Wu 1983) is found in the Yarlung Zhangbo valley between 93°E and 88°42′ E and in the lower Kyi Chu valley south of Lhasa. Only two of the surveyed sites cover several hectares and deserve to be called forest (appendix: 46, 57). *Juniperus convallium* is found on all exposures in these wide valleys (see Fig. 3). *Juniperus* stands on northern exposures may attain interlocking crowns, while on all other sites tree cover percentages range between 10% and 60%. On shady slopes the highest *Juniperus convallium* trees were found at 4,000 m in the east (appendix: 57), increasing in elevation westwards with the concomitant decrease of humidity to 4,280 m (appendix: 19). On sunny slopes *Juniperus convallium* ascends slightly higher and is found up to 4,200 m in the east (appendix: 57) and 4,580 m in the West (appendix: 19). The highest record so far is in the northern outskirts of Lhasa at 4,600 m on a steep, south-facing granite cliff (appendix: 41). The range of *Juniperus convallium* thus covers 400 km in an altitudinal belt between valley bottom (3,600–4,000 m) and 4,600 m.

Juniperus tibetica has a slightly greater distribution. This tree generally forms the upper tree line and is present in the more humid side valleys where it is obviously too cold for *Juniperus convallium* (see Fig. 3). The species is found primarily on sunny slopes, except where it occurs on steep, north-facing cliffs emerging from *Rhododendron-Salix* thickets.

The drought limit of *Juniperus* trees is difficult to assess. Lhaze (Fig. 2) has 263 mm summer rainfall and the last trees close to the climate station were destroyed during Cultural Revolution (appendix: 10). The nearest living *Juniperus* trees are *Juniperus tibetica* (appendix: 6–9, 11). The closely related *Juniperus indica* in arid environments of North Central Nepal, 320 km to the west of Lhaze, are found near climate stations with 200–250 mm rainfall (Miehe et al. 2002). These values compare well with reports of *Juniperus* woodlands in southeastern Spain (Freitag 1972) or the La Sal Mountains of Oregon

(Henning 1975). It is thus an open question whether the reforestation trials carried out in Lhasa (443 mm/a) would be successful further west. However there are seedlings and young trees found in the localities west of Lhaze showing clearly the vitality of the forests. From field evidence it seems implausible that the western *Juniperus* populations would regenerate into closed forests if firewood or incense extraction could be excluded. The tree stands, which survived so far in the west, suggest open forests with a crown cover between 10% and 30%. The most arid stands are heavily infested by mistletoe-like parasites (*Arceuthobium oxycedri*). Whether this is due to adverse climatic conditions or additional factors is not known.

The altitudinal range of *Juniperus* trees comprises vegetation units of the ''Vegetation of Xizang'' which had been translated according to the UNESCO classification (Mueller-Dombois and Ellenberg 1974) as ''scrub'', ''Cyperaceae-dominated pastures'' and ''alpine steppe''. The presence of trees in vegetation units classified as ''alpine'' is somewhat contradictary because ''alpine'' is by definition beyond the upper treeline and treeless due to growing conditions hostile for trees. The issue of forest islands in Tibet resembles the ''*Polylepis* problem'' in tropical alpine Paramo grassland of the Andes (Miehe and Miehe 1994; Kessler 2002). Considering the possible succession between the forest islands and the surrounding pasture types there is a clear divide between the stands in open dwarf shrublands (Fig. 2: vegetation units 12b, 12c, 13, 14, 18, 28, 32), on the one hand, and *Juniperus* groves on open soil patches surrounded by a closed cover of felty turf build of *Kobresia pygmaea*, on the other (Fig. 2: vegetation units 2–53, except 28, 30–32, see Figs. 4, 5). *Juniperus* stands in dwarf shrublands are common along the Yarlung Zhangbo and in the lower Kyi Chu. Figs. 4 and 5 show the quasi-erratic trees in desert-like commons. Taken together, the exclosure experiments near Lhasa, the field evidence of seedlings and *Juniperus* trees in the bird-dependent dispersal range of mother trees, and vegetation records in relict sites all reveal the potential of *Juniperus* forests with *Prunus mira* Koehne and *Buddleja crispa* as understorey trees and a larger number of shrubs and grasses. Pollen analysis close to the sites where reforestation trials were established provide evidence of *Juniperus* forests around Lhasa which were cleared around 4,600 yr BP and replaced by the current *Artemisia*-dominated pastures for the last 600 yr (Miehe et al. 2006). Thus the *Sophora moorcroftiana* heathlands of the Yarlung Zhangbo (Fig. 2: 18b), the *Stipa bungeana* steppe and the *Artemisia*-*Stipa capillata* steppe (Fig. 2: 28, 32) once were forests (or woodlands) and could be forest again because there are isolated tree stands on normal sites, including seedlings and young trees. These scenarios are given on the Digital Terrain Model of the Yamco Yumco basin (Fig. 6). The mapped area has a forest potential of 2,628 km^2 which is 22.4% of the total area of 11,730 km^2.

In the east (appendix: 32–37), especially in the headwaters of valleys of the eastern declivity of the

Fig. 5 Upper treeline ecotone at 4,850 m with multi-stemmed trees of *Juniperus tibetica* on the southern Yamco Yumco peninsula (28°54′ N/90°34′ E, Fig. 2: 51), southern exposure. August 1998. Photo G Miehe

Fig. 4 Isolated *Juniperus tibetica* tree of 3 m height, south of the Yarlung Zhangbo (29°09′ N/86°55′ E, Fig. 2: 7), 4,350 m on a southeast-facing ridge. This is a windward water deficit site near the drought line of *Juniperus*. March 1998. Photo G Miehe

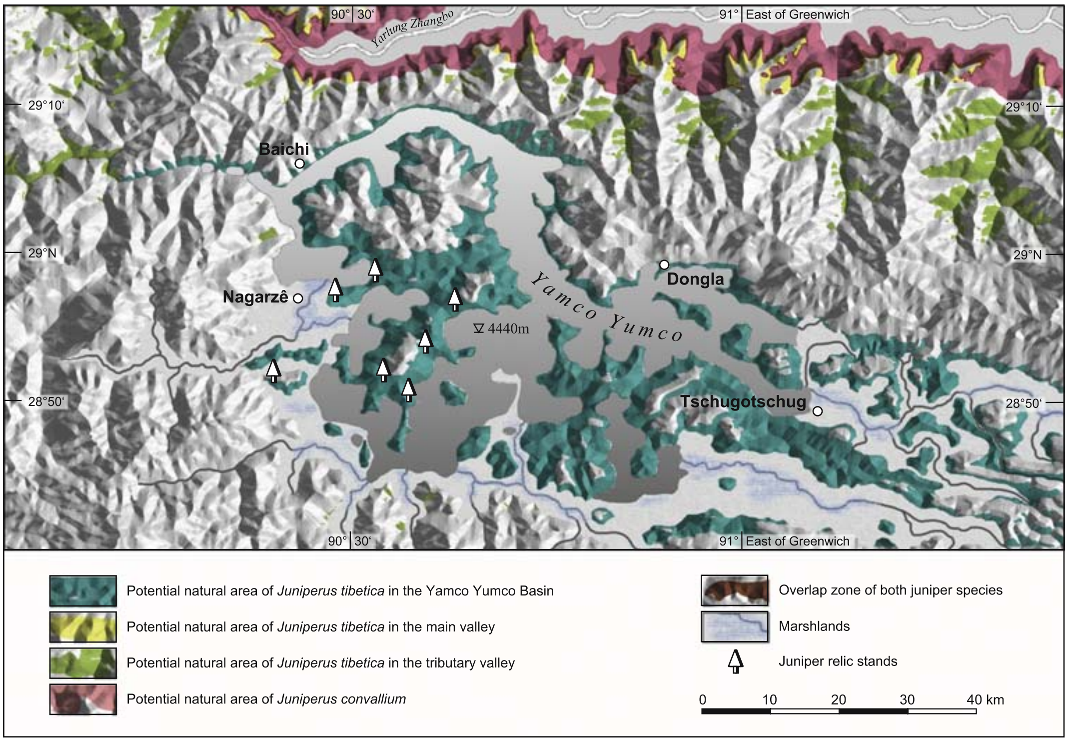


Fig. 6 Digital Terrain Modell of the Yamco Yumco Basin with potential natural *Juniperus* forests. The total surface is 11,730 km^2, the forest potential area is 2,628 km^2 (22.4%). Based on the *Juniperus* inventory, vegetation records, indicator values of plants, precipitation records and weather observations three types of potential *Juniperus* forests are designated: (1) the intramontane basin of the Yamco Yumco is assumed to have *Juniperus tibetica* forests in all exposures between 4,400 and 4,850 m; (2) in the Yarlung Zhangbo valley *Juniperus convallium* forests are mapped in all exposures up to 4,000 m; (3) this zone is followed by *Juniperus tibetica* to the upper treeline (4,600 m). Side valleys have only *Juniperus tibetica* forests. Draft M Will 2002. Software: GRASS (GIS, Microsoft Excel 1997)

Tibetan Plateau (Lhari, Fig. 7), the successional status is complex. The humidity is more than two times higher than in the western margin of an assumed *Juniperus* forest belt (Fig. 2: Lhari 707 mm/a), and *Juniperus* groves are distributed only spottily where the *Kobresia pygmaea* turf has been destroyed. The turf is part of the world's largest alpine ecosystem, covering ca. 450,000 km^2 in the south-eastern humid quarter of the Tibetan highlands (Miehe and Miehe 2000b). Exclosure experiments in Reting (appendix: 33, Miehe et al. 2004) have posed even more questions: Reting Forest, the largest and most sacred *Juniperus* forest of Tibet, has trees of 2 m dbh and 16 m in height, diffusely growing in a carpet-like felty turf of *Kobresia pygmaea*, the female trees surrounded by open soil. The forest is mentioned in the earliest documents of the monastery founded here in 1057 AD (P. Sörensen, pers. comm.); rejuvenation is poor most probably due to the low germination rate because trees are overmature. The *Kobresia pygmaea* mats are grazed yearround by yak but the grazing pressure is highest during winter. Two exclosure plots in those *Kobresia pygmaea* pastures revealed a total change from a Cyperaceae-dominated pasture resembling a golf course to a 50 cm tall *Stipa*

Fig. 7 South-facing *Juniperus tibetica* trees in the upper treeline ecotone (4,500 m) colonizing open soil patches in the felty turf of *Kobresia pygmaea* pastures of southeastern Tibet. East of Lhari, 30°38′ N/93°18′ E. July 2004. Photo G Miehe

grassland in only 4 yr time. Thus the relationship between the *Juniperus* trees and the Cyperaceae mats or grasslands is unknown. As *Kobresia pygmaea* has an altitudinal range reaching nearly 6,000 m a.s.l. (Miehe 1989) it is certainly less temperature demanding than *Juniperus* trees. It is therefore easy to assume that *Kobresia pygmaea* spread to cover the highlands with the present green golf course-like turf earlier than we could expect the re-migration of trees. In addition, *Kobresia pygmaea* establishes more readily and is wind dispersed, unlike *Juniperus*, which probably disperse more slowly because it is dispersed by birds (*Turdus merula*, *Babax Waddelli* Dresser; M. Karlstetter, pers. comm.). It is thus possible that *Kobresia pygmaea* turfs impeded the reforestation. The recent forest outposts of Reting and Lhari (see Fig. 6) have an upper treeline ecotone between 4,600 and 4,860 m. This raises the question why the *Kobresia*-covered highland pastures around Nagqu, with altitudes of 4,400–4700 m, are treeless. Rainfall (Nagqu 431 mm/a) would permit the development of forests and there are enough open sites where the *Kobresia pygmaea* turf has been destroyed. The explanation could be that winter temperatures are too cold for *Juniperus tibetica*: the Changtang, where Nagqu is situated, is exposed to catastrophic intrusions of cold air masses of the Siberian High during winter in contrast to the forest of Reting and Lhari which spread well above the cold air ponding effects of the tongue basins of those valleys (where the climate stations are situated). Moreover they are sheltered from cold air masses by mountain ranges toward the North.

Conclusion

Isolated *Juniperus* trees or forest islands in the pastoral desert environments of Southern Tibet are religious landmarks for Tibetan Buddhists. They are worshipped and protected. During the Cultural Revolution, many sacred trees were removed. Up until now, the natural resource potential of these forests has gone unrecognized in the scientific community. Vegetation surveys since 1984 have revealed more than 50 sites with vigorous *Juniperus* trees, which are not limited by water surplus, or any other obvious habitat factor. Additionally 13 sites are known from historical documents or are cited according to oral tradition that trees were destroyed during warfare or during the Cultural Revolution. The forest relicts are mostly dominated by a single tree species. Two *Juniperus* tree species have been found. *Juniperus convallium* is so far not known to occur in Southern Tibet but is present in an area of 400 km west of the current forest limits. The second tree species, *Juniperus tibetica*, stretches from the outer declivities of the Tibetan Plateau up to an area 650 km west of the present forest border. It is concluded that treeless areas between the current relicts were forested in the past and could become forested again if human interference were excluded. To prove this assumption experimental reforestation trials on southern slopes above Lhasa have been carried out since 1999 with non-irrigated plantations of nursery-raised seedlings of *Juniperus convallium, Juniperus tibetica* and *Cupressus gigantea*. Sampling after three years indicated survival rates of nearly 100%. The highest trees of *Juniperus tibetica* were found at an elevation of 4,860 m. Except the trees growing in rockwalls all relict stands are heavily grazed and seedlings or young trees survive only where grazing and trampling is light. A correlation with tree stands and rainfall data of the nearest climate stations reveal that the drought line of *Juniperus* forests in Southern Tibet is approximately 200–250 mm/a. Vegetation records allow the reconstruction of three potential forest types (see Fig. 3). Under more arid conditions, even northern exposures have *Juniperus* forests but in general *Juniperus* are found on southern exposures. In the eastern part of the distribution area and in the cloudy cooler side valleys, *Betula* forests form the upper treeline ecotone of the shady slope, or at least have the potential to do so. As *Betula* is heavily browsed, the reconstruction of the natural range of *Betula* forests is even more difficult than *Juniperus* because there are very few remaining relicts. The first pollen diagram from the area (Schlütz in Miehe et al. 2006) dates human use, at least for the area of Lhasa, to as early as 4,600 yr BP: Pollen of ruderal weeds and cereals indicative of human use are synchronous with the decline of *Juniperus* pollen. Human impact increased during the last 600 years, supporting the evolution of the present degraded pastures.

It remains unknown when forests began to spread following the Last Glacial Maximum, and from where. Altitudes, relief and current climatic conditions make it most probable that the lower Yarlung Zhangbo valley downstream of 93°E is the nearest forest refugium.

In more humid Eastern Tibet, *Juniperus* are strictly bound to open soil patches surrounded by a dense, felty Cyperaceae mat of *Kobresia pygmaea*. It is unknown whether *Juniperus* colonize the open soil patches after the removal of the felty turf or whether the *Kobresia pygmaea* pastures colonized space that had been cleared from forests. It cannot be excluded that this turf cover impeded the re-migration of forests to the plateau in the early Holocene.

Most of the forests are protected for religious reasons, and since 2002 have acquired the official status of protected area (under the responsibility of the Forestry Bureau of the Xizang Autonomous Region). Despite this, they are prone to extinction due to lack of regeneration stemming from low viability of the embryos, low germination rates, genetic drift following fragmentation and due to grazing and trampling impacts. Timber extraction or fire wood use and in some sites even incense collecting is now banned and widely obeyed. All isolated *Juniperus* sites of Southern Tibet are threatened; this is especially true of the few populations of *Juniperus convallium* and the western-most outposts of *Juniperus tibetica*. The IUCN information about the status as given in Farjon (2005) is misleading.

As the lack of fire wood is a severe limitation of rural economy in Southern Tibet, it is necessary to introduce forest rehabilitation measures with indigenous forest species that do not depend on the decreasing supply of irrigation water (Miehe et al. 2003). The current inventory could be used as a data base for GIS modelling of potential reforestation areas which are currently not believed to have natural forests.

Acknowledgements The inventory was carried out within the framework of the Lhasa-Marburg University partnership programme supported since 2003 by the VW Foundation, in cooperation with the Tibet Plateau Biology Institute of Lhasa (1995–1999) and Institutes of the Chinese Academy of Sciences in Xining, Lanzhou and Chengdu (1999–2004). The German Research Council generously supported all expeditions. Christiane Enderle (Marburg) prepared maps and graphs with her usual elaborateness. Jürgen Böhner (Hamburg) kindly provided data of Transeau ratios. Two unknown reviewers helped us improving the article considerably.

Appendix

1. Tangra Yumco-basin, 30°30′–31°30′ N/86°20′–86°50′ E, 4.500–4.800 m: According to local oral tradition (Nam Thag, Sheshik gompa, pers. comm., Sept. 9th 2003) the *Juniperus* forests of the lake basin were destroyed by fire during warfare ca 650 AD. Actually no remains, except of two *Juniperus* trees on the eastern bank of the lake at Kisum (31°06′ N/86°48′ E, 4,590 m), ca. 2.5 m, single-stemmed, 0.4 m Dbh (Dorgeh, pers. comm., April 6th 2006). No *Juniperus pingii* v. *wilsonii*. No seedling data.
2. Langma Chu near Tagtse, 29°14′ N/86°15′ E, 4,850 m, S-exp.: *Juniperus tibetica*, ♂ and ♀,30 cm Dbh, 3.5 m. Sacred. Known as westernmost *Juniperus*-tree along the Yarlung Zhangbo. More multi-stemmed individuals 0.8–2 m on the same slope. No seedling data.
3. Cliffs of the Yarlung Zhangbo gorge East of Tagtse, 29°12′ N/86°16′ E, 4,850 m, S-exp.: 6 *Juniperus tibetica* ♂ and ♀, 0.6–2 m, multi-stemmed, partly lopped. No seedling data.
4. Semik. Cliffs of the Yarlung Zhangbo gorge between Tagtse and Chung Riwoqe, 29°93′ N/86°18′ E, 4,200–4,600 m, S-exp.: *Juniperus tibetica* trees in steep rocks. Exploited for incense from villagers of Tagtse and Chung Riwoqe. No seedling data.
5. Chung Riwoqe, 29°11′ N/86°36′ E, 4,180 m: *Juniperus*-forests according to the pilgrimage record of Katoxitu of 1920 (K.H. Everding, pers. comm., 1997). Actually no remains.
6. Lheding, 29°13′ N/86°52′ E, 4300–4950 m, W-S-E-exp.: above the gompa and on neighboring slopes: *Juniperus tibetica* ♂ and ♀, open forest, multi-stemmed, to 3.5 m; at the upper limit gradually dwarf (0.5 m) with *Juniperus pingii* v. *wilsonii*. Seedlings.
7. South bank of the Yarlung Zhangbo East of Dobe, 29°09′ N/86°55′ E, 4,350 m, SE-exp. on ridge: solitary *Juniperus tibetica* tree, ca. 3 m (see Fig. 4). No seedling data.
8. Yarlung Zhangbo gorge between 87°07′ and 87°12′ E, 4,100–4,650 m, on both banks of the river, but mostly on sunny exposures of the northern bank. Best preserved tree stands around the ruins of Chugdö gompa (29°09′ N/87°09′ E, 4,250–4,650 m): *Juniperus tibetica*, ♂ and ♀,

multi-stemmed, 6 m. Mostly resprouting globular bushes 2–3 m. Seedlings.

9. Limestone scree slope above La'ang Co, 29°19′ N/87°23′ E, 4,440–4,600 m, S-exp.: *Juniperus tibetica*, ♂ and ♀, several multi-stemmed lopped bushes up to 2.5 m in *Lonicera-Rosa* thickets. Several stunted dwarf *Juniperus* trees on inaccessible rock-walls. No seedling data.
10. Kjelde near Lhaze: Northern bank of the Yarlung Zhangbo, 29°08′ N/87°35′ E, 4,034 m, S-exp.: Single *Juniperus* tree, close to the destroyed Tachung gompa, blasted and eradicated 1959, according to local informant (Sept. 18th 2003).
11. Sim Gompa, 29°06′ N/87°57′ E Gr., 4,300–4,750 m, S-exp.: *Juniperus tibetica*, ♂ and ♀, 5 m, max. 0.4 m Dbh. Open forest and resprouting bushes. Seedlings. Largely depleted for road construction purposes around 1970 (according to local informants, Sept. 18th, 2003).
12. Sakya monastery, 28°54′ N/88°01′ E, 4,350 m, S-exp.: Several *Juniperus* trees destroyed during the Cultural Revolution (according to local informants, August 30th, 2005).
13. Intramontane basin of Zhetongmen, 29°23′ N/88°10′ E, 4,020 m, S-exp.: At Entsang several *Juniperus* trees were destroyed with the gompa during Cultural Revolution (local informant, August 30th, 2005).
14. Northern bank of the Yarlung Zhangbo,east of Xigaze, above Tuge, 28°21′ N/88°42′E, 3,980 m, S-exposed on granite crest 80 m above the floodplain: Westernmost record of *Juniperus convallium*, 3 monocious trees 4–6 m, single and multi-stemmed, max. 0.5 m Dbh. Seedlings, no drought damages. Remains of a sacred forest of more than 100 trees, largely destroyed during Cultural Revolution (according to local informant, August 31st, 2005).
15. Rindu, North of Namling, 30°03′ N/89°06′ E, 4,410–4,470 m, S-exp.: *Juniperus tibetica*, ♂ and ♀, 2.5–4 m, open forest and resprouting bushes, young trees. No seedlings. Known as the northernmost *Juniperus* trees in this valley.
16. Northern bank of the Yarlung Zhangbo gorge near Tschewa, 29°18′ N/89°44′ E, ca. 4,200 m, NW-exp.: Several solitary *Juniperus* trees (4–8 m), at least partly religiously preserved. No seedling data.
17. Rimphu-valley, 29°12′ N/89°52′ E, 3,970 m: *Juniperus tibetica*, 8 m, 0.5 m Dbh. Dead tree in yard, 1995 crown damaged by storm. Local traditions tell about a *Juniperus* forest in the upper Rimphu valley, which was burnt (local informant, August 12th 1999).
18. Southern bank of the Yarlung Zhangbo gorge, 29°19′ N/89°56′ E, 3,820–4,000 m. Northern exposures: *Juniperus convallium*, ♂ and ♀, multi-stemmed trees 2–3 m, resprouting bushes and obviously young *Juniperus* (<0.5 m). Inaccessible rocky slopes have scattered stands of *Juniperus convallium* trees. No seedling data.
19. Southern (29°20′ N/89°57′ E) and northern (29°20′ N/89°59′ E) bank of the Yarlung Zhangbo gorge W of Nyemo: *Juniperus convallium* shrubs in leeward southern exposures until 4,580 m. Trees 6 m, 0.5 m Dbh, 3,980 m, S-exp. next to ruins of gompa. On the southern bank *Juniperus convallium* and *Juniperus tibetica* until 4,280 m; *Juniperus convallium* trees and shrubs only in easterly and westerly exposures, northerly facing stands only on very exposed rocky ridges. *Juniperus tibetica* only in northern exposures. *Juniperus tibetica* trees between 2 and 4 m, mostly multi-stemmed on sunny exposures between 4,500 and 4,850 m. Highest *Juniperus tibetica* tree of 4 m in 4,850 m. Seedlings on both banks.
20. Yarlung Zhangbo gorge West of Nyemo, 29°22′ N/90°09′ E, 4,080 m, SW-facing: 6 isolated *Juniperus convallium*, ♂ and ♀, 1.5–4 m, max. 0.3 m Dbh, known to locals as ‘‘seven brothers’’. Seedlings.
21. Nyemo, 29°24′ N/90°10′ E, 3,850 m, in the yard of a farmhouse: *Juniperus convallium*, 0.3 m Dbh, 6 m, brought as a small tree from the Yarlung Zhangbo gorge and planted in the yard. No seedling data. *Juniperus* groves with deer are reported from the upper Nyemo valley as late as in 1947 (fide Aufschnaiter in Brauen 1983).
22. Yarlung Zhangbo gorge East of Nyemo, 29°20′N/94°14′ E, 3,720 m; boulder of ca. 30 m^3 at the southern bank of the river: *Juniperus convallium* ca 1 m, growing on top of the boulder. No seedling data.

23. Tunda valley, 29°22′ N/90°20′ E, 4,080 m: *Juniperus tibetica*, 12 m, 0.3 m Dbh in a yard. Brought as a small tree from Loura. 29°24′ N/90°23′E, 4,350 m, S-exp.: Single *Juniperus* tree close to the gompa, destroyed by blasting during the Cultural Revolution. Tunda is famous for its incense products since 700 years. The main ingredient is *Juniperus* which was taken until 1984 totally from this valley. For the traditionally grounding technique in water mills logs need to have a diametre of at least 20 cm. No seedling data.
24. Loura: Northern tributary valley of the Yarlung Zhangbo gorge east of Tunda, 29°20′ N/90°21′ E, 4,350–4,500 m, SE-exp.: *Juniperus tibetica*, ♂ and ♀, single-stemmed trees up to 5 m and coppice regrowth or resprouting trees 2.5–4 m, juvenile specimens of 50 cm. Seedlings.
25. Above Chuba, 29°28′ N/90°43′ E, 4100–4300 m, all exposures: *Juniperus tibetica*, ♂ and ♀; single, mostly multi-stemmed trees and bushes, 1.5–3 m, in open woodlands. Seedlings.
26. Nienang monastery, 29°45′ N/90°41′ E, 4,340–4,450 m, S-exp.: *Juniperus tibetica*, ♂ and ♀, 12 m, max. 0.4 Dbh, trees and resprouting bushes around the monastery. Seedlings.
27. Western tributary of the Tolung Chu, 29°44′ N/90°42–43′ E, 4,050–4,400 m, southern and eastern exposures: *Juniperus tibetica*, ♂ and ♀, 0.5–1 m, trees and resprouting bushes. No seedling data.
28. E of Tsurphu monastery N of 29°44′ N/90°44′ E, ca. 4,500–4,700 m relic trees on S-facing slopes: *Juniperus tibetica*. No seedling data.
29. Upper Tolung Chu near Yangpachen, 30°03′ N/90°35′ E, 4,300 m, S-exp.: *Juniperus tibetica*, tree, 3 m. No seedling data.
30. East of Yangpachen, 29°50′ N/90°21′ E, 4,300 m, SW-exp.: *Juniperus* grove was cut for military purposes in the 1960s and regenerates with coppice regrowth. No seedling data.
31. Nindung Xiang, 30°23′ N/90°54′ E, 4,250–4,300 m, southern exposures: *Juniperus tibetica*, ♂ and ♀, trees up to 3 m, max. 0.3 m Dbh. Open tree stands in scree of roche moutoneé, sacred forest depleted during Cultural Revolution. No seedlings.
32. Western branch of the upper Kyi Chu (Rong Chu) between 30°15′ N/91°17′ E and 30°20′ N/91°04′ E from the foot of the slope (4,100 m) to the crest in southerly exposures: Open *Juniperus tibetica* forests and coppice regrowth after timber extraction, with interlocking crowns in undisturbed sites under religious protection (Tsowa gompa, 30°16′ N/91°10′ E, above 4,150 m). Seedlings.
33. Upper Kyi Chu catchment between 30°15′–30°24′ N and 91°23–91°43′ E, 4,200–4,860 m, preferably in southern exposures, extending to rocky easterly and westerly facing slopes, even on exposed rock cliffs of northern exposures: *Juniperus tibetica*, ♂ and ♀, trees, up to 16 m, max. 2.5 m Dbh (in the sacred forest of Reting monastery, 30°18′ N/91°31′ E) and resprouting bushes in non-protected areas. Seedlings. Ludlow (1951) reports of deer, bears and leopards in great numbers in this forest.
34. Middle Kyi Chu catchment between 30°11′ N/91°21′ E and 30°00′ N/91°53′ E, 4,000–4,800 m, southern exposures: *Juniperus tibetica*; several sacred groves above villages and lopped bushes in the commons around the preserved groves. Largest groves above Barza gompa (30°05′ N/91°33′ E) and Shoten gompa (30°08′ N/91°33′ E). Seedlings.
35. Drigung in the eastern upper Kyi Chu catchment between 30°00′ N/92°02′ E and 30°06′ N/92°20′ E, 4,300–4,600 m; southern exposures: *Juniperus tibetica*, ♂ and ♀, up to 7 m, 0.3 m Dbh, scattered tree stands and lopped bushes. The sacred forest around Drigung monastery was destroyed during the Cultural Revolution. Resprouting multi-stemmed trees up to 2 m. Seedlings.
36. Upper Nyang Chu, 29°54′ N/92°27′ E, 4,470 m, S-exp.: *Juniperus tibetica*, ♂ and ♀, up to 0.3 m Dbh, up to 3 m, open tree stands. Westernmost forest in the upper Nyang Chu catchment. Seedlings.
37. Northern bank of the Nyang Chu upstream of 29°57′ N/92°51′ E: *Juniperus tibetica*, ♂ and ♀, up to 7 m, up to 0.4 m Dbh. Numerous open forests on sunny slopes of winter pastures. On northern exposures confined to cliffs. Upper treeline ca. 4,600 m. Seedlings.
38. Gyama Chu, 29°42′ N/91°40′ E, 4,200 m: *Juniperus tibetica*, ♂ and ♀, 5 and 6 m, 0.3 m

Dbh, trees on hilltop and on SW-facing slope near gompa. No seedling data.

39. East bank of the Kyi Chu, Dako, 10 km NE of Meldro Gungkar, 29°53′ N/92°20′ E, 4.050 m: 2 *Juniperus*-trees. According to a local informant a greater number of trees were removed during Cultural Revolution. No seedling data.
40. Intramontane basin of Phempo: Sacred *Juniperus* trees and groves were destroyed together with the gompas during Cultural Revolution at Nalendra gompa (29°52′ N/91°07′ E, 3,900 m), and Ragme Jamkang (29°54′ N/91°02′ E, 4,000 m), according to local informant, Sept. 4th, 2005.
41. Lhasa, above Porong Ka and Chupsang monastery, 29°43′ N/91°07′ E, ca. 4,600 m S-exp.: 3 *Juniperus tibetica* and *J. convallium* (1–3 m tree + shrub) in steep cliff. The foundation legend of Tashi Tsöling monastery (4,050 m, above Chupsang) says that the monastery was situated amongst *Juniperus* trees. The last individuals are said to be cut during Cultural Revolution (head of Tashi Tsöling monastery, pers. comm. 1997). Aufschnaiter (in Brauen 1983) mentions a large *Juniperus* tree trunc W of Sera monastery in the late 40's, obviously close to the locality of 41. No seedling data.
42. Tschalö, south of Lhasa, 29°38′ N/91°00′ E, 3,640 m: *Juniperus tibetica*, ♀, 15 m, 2 m Dbh, partly decayed, fenced and religiously worshipped. The tree most probably reaches the ground water in the gravel terrace of the Kyi Chu. No seedlings.
43. Eastern bank of the Kyi Chu, south of Lhasa, 29°35′ N/91°07′ E, 3,680 m, W-exp.: 2 *Juniperus convallium* trees in steep cliff. No seedling data. Nyima Thang (29°30′ N/91°07′ E, 3,900–4,200 m: Several *Juniperus tibetica* trees (12 m, up to 0.5 m Dbh), religiously protected. No seedling.
44. Upper Datse valley 29°33′ N/91°24′ E, 4,200 m S-exp.: *Juniperus tibetica* trees, 4 m, 0.2 m Dbh, trees near houses and in steep rock walls. No seedling data.
45. Chungse monastery, 29°27′ N/91°01′ E, 4,300–4,480 m, southern exposure: *Juniperus tibetica* ♂ and ♀, numerous caespitose resprouters from tree-stumps cut during Cultural Revolution around the monastery. Seedlings.
46. Chirong, 29°22′ N/90°53′ E, 3,600–4,200 m (crest), all exposures: *Juniperus convallium*, above 4,100 m in northern exposures *Juniperus tibetica*, ♂ and ♀, 5 m, up to 0.3 m Dbh. Open forest, best preserved above the village as sacred, partly cleared during Cultural Revolution. Seedlings. *Juniperus tibetica*, ♂ and ♀, single and multistemmed trees up to 0.3 m Dbh and 4 m on N-exposed slope above 3,900 m south of the village. Seedlings.
47. Nagarze, 28°58′ N/90°24′ E, 4,450 m, SE-exp.: Dead multi-stemmed *Juniperus* tree, 9 m, near to the gompa.
48. Tsamchü, 3 km east of Nagarze, 28°59′ N/90°26′ E, 4,400–4,650 m; ESE- to WNW-exposed: *Juniperus tibetica*, ♂ and ♀, open forest, single and multi-stemmed, max. 0.5 m Dbh, 3–6 m, at the upper limit gradually dwarf (highest caespitose *Juniperus tibetica* shrubs 0.3 m, 4,750 m). Largest sacred forest south of the Yarlung Zhangbo, partly cleared during Cultural Revolution. Seedlings.
49. Samding monastery, 6 km east of Nagarze, 28°59′ N/90°28′ E, 4,450–4,550 m: Several *Juniperus tibetica* trees below the monastery, 2–5 m, 0.2–0.4 m Dbh. Seedlings.
50. Atsha, central peninsula of the Yamco Yumco, 28°58′ N/90°35′ E, 4,500–4,600 m, on open ridge: Several lopped *Juniperus tibetica*-trees. No seedling data.
51. Southern peninsula of the Yamco Yumco, 28°47–50′ N/90°30–48′ E, 4,450–4,850 m, all exposures (even north!): *Juniperus tibetica* ♂ and ♀, single and multistemmed trees (up to 0.4 m Dbh, up to 4 m), open woodlands, upslope gradually caespitose with *Juniperus pingii*—shrubs (see Fig. 5). Seedlings in all exposures.
52. Hills north of Taglung, 28°52′ N/90°22′ E, 4,400–4,600 m, S- and SW-exp.: *Juniperus tibetica*, 0.5–3 m, trees and scattered resprouting bushes. No seedling data. In Taglung: *Juniperus tibetica*, multi-stemmed tree, 5 m, in yard.
53. Upper Drip Chu, 29°01′ N/91°05′ E, 4400 m, E-exp.: The last four *Juniperus* trees were destroyed during a landslide in 1999, according to local informants (Sept. 19th, 2002). It is commonly believed in the valley that in the unknown past there were *Juniperus* forests below Drip (north of 29°00′ N/91°05′ E).

54. Chimpu monastery above Samye, 29°22′ N/91°32′ E, 4,070–4,600 m, S-exp.: *Juniperus tibetica*, ♂ and ♀, trees max. 6 m, 0.4 m Dbh, resprouting bushes around the monastery. Seedlings.
55. Hills south of Zetang: During warfare of the 6th century AD. soldiers hid in (*Juniperus*) forests near Pyingiba. Forests were burnt down near Thangboche before the foundation of the monastery (11th century AD.) (P. Sörensen, pers. comm.).
56. Northern bank of the Yarlung Zhangbo above Pamtschü, 29°16′ N/91°57′ E, 3,650–4,600 m: *Juniperus convallium* ♂ and ♀, up to 4,100 m, where *Juniperus tibetica* continues up to the treeline at c. 4,600 m, ♂ and ♀. Trees of 0,4 m Dbh and up to 9 m only around the Densatil monastery (4,450 m). Seedlings.
57. Northern bank of the Yarlung Zhangbo above Halung, 29°18′ N/92°08′ E, 3,800–4,770 m, all exposures: *Juniperus convallium*, ♂ and ♀, lopped trees (up to 3 m, up to 0.3 m Dbh) in open woodlands, all exposures between 3,800 and 4,000 m; on southerly slopes up to 4,200 m. *Juniperus tibetica* between 4,050 and 4,770 m with single and multistemmed trees (up to 5 m, up to 0.3 m Dbh) on all exposures seemingly too dry for *Betula* and dense *Salix-Sibiraea* scrub. Seedlings.
58. Yarlung Zhangbo bridge at Sangri, 29°14′ N/92°00′ E, 3,560 m: *Juniperus convallium*, ♀, tree, 0.3 m Dbh, 6 m, in reach of the ground water. Believed of having been planted as sacred tree near the bridge. No seedlings.
59. Intramontane basin of Qusum, 29°04′ N/92°10′ E, 3.900 m: several isolated *Juniperus tibetica* trees mostly in yards. No seedling data.
60. South of Qusum, 29°02′ N/92°12′ E, 4,150 m: *Juniperus tibetica*-tree, 8 m, 0,3 m Dbh, on cliff of river terrace. No seedling.
61. Northern tributary of the Subansiri, 28°36′ N/92°32′ E, 4,070 m, all exposures: *Juniperus tibetica*, ♂ and ♀, single-stemmed trees, 3–6 m, 0.2–0.5 m Dbh. Seedlings.

References

Aldenderfer M, Zhang Y (2004) The prehistory of the Tibetan Plateau to the seventh century A.D.: perspectives and research from China and the west since 1950. J World Prehistory 18:1–55

An C-B, Feng Z-D, Barton L (2006) Dry or humid? Mid-Holocene humidity changes in arid and semi-arid China. Quaternary Sci Rev 25:351–361

Institute of Geography, Chinese Academy of Sciences (1990) Atlas of Tibet. Science Press, Beijing (in Chinese)

Blyakharchuk TA, Wright HE, Borodavko PS, Knaap VDWO, Ammann B (2004) Late Glacial and Holocene vegetational changes on the Ulagan high mountain plateau, Altai Mountains, Southern Siberia. Palaeogeogr Palaeoclimatol Palaeoecol 209:259–279

Böhner J (1996) Säkulare Klimaschwankungen und rezente Klimatrends Zentral- und Hochasiens. Göttinger Geographische Abhandlungen 101

Böhner J, Lehmkuhl F (2005) Climate and environmental change modelling in Central and High Asia. Boreas 34:220–231

Brauen M (1983) Peter Aufschnaiter. Sein Leben in Tibet. Steiger, Innsbruck

Bräuning A (1999) Zur Dendroklimatologie Hochtibets während des letzten Jahrtausends. Dissertationes Botanicae 312, Stuttgart

Campo EV, Cour P, Hang S (1996) Holocene environmental changes in Bangong Co basin (Western Tibet). Part 2: the pollen record. Palaeogeogr Palaeoclimatol Palaeoecol 120:49–63

Chandra Das S (1902/1988) Lhasa and Central Tibet. Mehra Offset Press, Delhi

Chayet A (1994) Art et Archéologie du Tibet. Picard, Paris

Chen S, Liu Y, Thomas A (2006) Climatic change on the Tibetan Plateau: potential evapotranspiration trends from 1961–2000. Climate Change 76:291–319

Da N, Keng H, Shan F (1989) A preliminary investigation on the vegetational and climatic changes since 11,000 years in Qinghai Lake—an analysis based on palynology in core QH 85/14C. Acta Bot Sinica 31:803–814

Farjon A (2005) A monograph of Cupressaceae and Sciadopitys. Royal Botanic Gardens, Kew

Freitag H (1972) Die natürliche Vegetation des südostspanischen Trockengebietes. Bot Jahrb 91:147–308

Frenzel B (1994) Über Probleme der holozänen Vegetationsgeschichte Osttibets. Göttinger Geographische Abhandlungen 95:143–166

Frenzel B, Pecsi M, Velichko AA (1992) Atlas of palaeoclimates and palaeoenvironments of the Northern Hemisphere. Borntraeger, Stuttgart

Fu D-X, Xu T-W, Feng Z-Y (2000) The ancient carbonized barley (*Hordeum vulgare* L. var. *nudum*) kernel discovered in the middle Yalu Tsangpo river basin in Tibet. Southwest China J Agricul Sci 13:38–41

Gunin PD, Vostokova EA, Dorofeyuk NI, Tarasov PE, Black CC (1999) Vegetation dynamics of Mongolia. Geobotany 26. Kluwer, Dordrecht

Henning I (1975) Die La Sal Mountains, Utah. Ein Beitrag zur Geoökologie der Colorado-Plateau-Provinz und zur vergleichenden Hochgebirgsgeographie. Abhandlungen Akademie der Wissenschaften und Literatur. Math.-Naturwiss. Klasse, Jahrgang 1975.2

Henning I (1994) Hydroklima und Klimavegetation der Kontinente. Münstersche Geographische Arbeiten 37

Herzschuh U, Kürschner H, Mischke S (2006) Temperature variability and vertical vegetation belt shifts during the last ~50,000 years in the Qilian Mountains (NE margin of the Tibetan Plateau, China). Quaternary Res 66:133–146

Jäger EJ (2005) The occurrence of forest plants in the desert mountains of Mongolia and their bearing on the history of the climate. Biol Res Mongolia 9:289–298

Kaiser K, Miehe G, Schoch WH, Zander A, Schlütz F (2006) Relief, soil and lost forests: late Holocene environmental changes in Southern Tibet under human impact. Zeitschrift Geomorphologie, Suppl 142:149–173

Kessler M (2002) The ''*Polylepis* problem'': where do we stand? Ecotropica 8:97–110

Kuhle M, Jacobsen J-P (1988) On the geoecology of Southern Tibet. GeoJournal 17:597–613

Lauer W, Rafiqpoor MD, Frankenberg P (1996) Die Klimate der Erde. Erdkunde 50:275–300

Ludlow F (1951) The birds of Kongbo and Pome, SE Tibet. The Ibis 93:547–578

Miehe G, Miehe S (1994) Zur oberen Waldgrenze in tropischen Gebirgen. Phytocoenologia 24:53–110

Miehe G, Miehe S (2000a) Comparative high mountain research on the treeline ecotone under human impact. Erdkunde 54:34–50

Miehe G, Miehe S (2000b) Environmental changes in the pastures of Xizang. Marburger Geographische Schriften 135:282–312

Miehe G (1986) The ecological law of ''relative habitat constancy and changing biotope'' as applied to multizonal high mountain areas. Proceed. Intern. Symposium Mountain Vegetation. Science Press, Beijing, pp 56–59

Miehe G (1989) Vegetation patterns on Mt. Everest as influenced by monsoon and föhn. Vegetatio 79:21–32

Miehe G, Miehe S, Schlütz F (2002) Vegetationskundliche und palynologische Befunde aus dem Muktinath-Tal (Tibetischer Himalaya, Nepal). Ein Beitrag zur Landschaftsgeschichte altweltlicher Hochgebirgshalbwüsten. Erdkunde 56:268–285

Miehe G, Miehe S, Huang J, Otsu T (1998) Forschungsdefizite und -perspektiven zur Frage der potenziellen natürlichen Bewaldung in Tibet. Petermanns Geographische Mitteilungen 142:155–164

Miehe G, Miehe S, Koch K, Will M (2003) Sacred forests in Tibet. Using Geographical Information Systems for forest rehabilitation. Mountain Res Develop 23:324–328

Miehe G, Miehe S, Koch K (2004) Towards the green belt in southern Xizang. Initial results of an applied research project to rehabilitate degraded rangelands. Proceed. Intern. Workshop Strategic Innovations for improving pastoral livelihoods in the Hindu Kush-Himalayan highlands. ICIMOD (Kathmandu), pp 48–50, 61–64

Miehe G, Miehe S, Opgenoorth L, Cermak J, Schlütz F, Jäger EJ, Wesche K, Samiya R (Ms. in review Palaeo 3): Mountain forest islands and Holocene forest retreat in Central Asian deserts. A case study from the South Gobi Altay. Mongolia

Miehe G, Miehe S, Schlütz F, Kaiser K, La D (2006) Palaeoecological and experimental evidence of former forests and woodlands in the treeless desert pastures of Southern Tibet (Lhasa, A.R. Xizang, China). Palaeogeogr Palaeoclimatol Palaeoecol 242:54–67

Miehe G, Miehe S, Vogel J, La Duo, Sonam Co (Ms. in review Mountain Research Development) Highest treeline of the northern Hemisphere found in Southern Tibet at 4,900 m

Miehe G, Winiger M, Böhner J, Zhang Y (2001) The climatic diagram map of High Asia. Purpose and concepts. Erdkunde 55:94–97

Mueller-Dombois D, Ellenberg H (1974) Aims and methods of vegetation ecology. John Wiley and Sons, New York

Pachur H-J, Altmann N (2006) Die Ostsahara im Spätquartär. Ökosystemwandel im größten hyperariden Raum der Erde. Springer, Berlin

Petit-Maire J, Bouysse P (2002) Cartes des environnements du monde pendant des deux derniers extrêmes climatiques. 1:50 M. Aix-en-Provence

Ren G (2000) Decline of the mid- to late Holocene forests in China. J Quaternary Sci 15:273–281

Schlütz F (1999) Palynologische Untersuchungen über die holozäne Vegetations-, Klima- und Siedlungsgeschichte in Hochasien und das Pleistozän in China. Dissertationes Botanicae 315

Song M, Zhou C, Hua Q (2004) Distributions of dominant tree species on the Tibetan Plateau under current and future climate scenarios. Mountain Res Develop 24:166–173

Tang LY, Shen CM (1996) Late Cenocoic vegetational history and climatic characteristics of Qinghai-Xizang Plateau. Acta Micropalaeontologica Sinica 13:321–337

Thelaus M (1992) Some characteristics of the mire-development in Hongyuan County, eastern Tibet Plateau. Proceed. 9th Intern. Peat Congress 1. Uppsala, pp 334–351

Thomas A, Chen S (2002) Landwirtschaft und klimatische Trends im zentralen Yarlung Tsangpo-Tal, Tibet. Erdkunde 56:371–384

Walter H, Walter E (1953) Einige allgemeine Ergebnisse unserer Forschungsreise nach Südwest-Afrika 1952/53: Das Gesetz der relativen Standortkonstanz; das Wesen der Pflanzengemeinschaften. Ber Deutsch Bot Ges 66:228–236

Wesche K, Ronnenberg K, Hensen I (2005) Lack of sexual reproduction in dry mountain steppe populations of the clonal shrub *Juniperus sabina* L. in Southern Mongolia. J arid environ 63:390–405

Wu C (1983–1987) Flora Xizangica. 5 vols. Science Press, Beijing (in Chinese)

Yu J (2004) Pollen based reconstruction of late Pleistocene and Holocene vegetation and climatic changes of Yang Lake area, Tibet. Chinese Bull Bot 21:91–100

Yu G, Tang L, Yang X, Ke X, Harrison SP (2001) Modern pollen samples from alpine vegetation on the Tibetan Plateau. Global Ecol Biogeogr 10:503–519

Zhang D, Li SH (2002) Optical dating of Tibetan hand- und footprints: an implication for the palaeoenvironment of the last glaciation of the Tibetan Plateau. Geophys Res Lett 29(16):1–3

Zhang J (1988) Vegetation of Xizang. Science Press, Beijing (in Chinese)

Zhang Q, Chiang T, Miehe G, Schlütz F, Liu J, Abbott RJ (2005) Phylogeography of the Qinghai-Tibetan Plateau endemic *Juniperus przewalskii* (Cupressaceae) inferred from chloroplast DNA sequence variation. Mol Ecol 14:3513–3524

Paper II

Quaternary Science Reviews 28 (2009) 1539–1554

Contents lists available at ScienceDirect

Quaternary Science Reviews

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

Charcoal and fossil wood from palaeosols, sediments and artificial structures indicating Late Holocene woodland decline in southern Tibet (China)

Knut Kaiser[a,*], Lars Opgenoorth[a], Werner H. Schoch[b], Georg Miehe[a]

[a] *Faculty of Geography, University of Marburg, Deutschhausstrasse 10, D-35032 Marburg, Germany*
[b] *Laboratory of Quaternary Woods, Unterruetistrasse 17, CH-8135 Langnau, Switzerland*

ARTICLE INFO

Article history:
Received 14 March 2008
Received in revised form
23 February 2009
Accepted 26 February 2009

ABSTRACT

Charcoal and fossil wood taken from palaeosols, sediments and artificial structures were analysed in order to evaluate the regional pedoanthracological potential and to obtain information on Holocene environmental changes, particularly on possible past tree occurrences in southern Tibet. This research was initiated by the question to what extent this area is influenced by past human impact. Even recent evaluations have perceived the present treeless desertic environment of southern Tibet as natural, and the previous Holocene palaeoenvironmental changes detected were predominantly interpreted to be climate-determined. The material analysed – comprising a total of 53 botanical spectra and 55 radiocarbon datings from 46 sampling sites (c. 3500–4700 m a.s.l.) – represents the largest systematically obtained data set of charcoal available from Tibet so far. 27 taxa were determined comprising trees, (dwarf-) shrubs and herbs as well as grasses. The predominant tree taxa were *Juniperus*, *Hippophae*, *Salix* and *Betula*. According to their present-day occurrence in the region, the genera *Juniperus* and *Hippophae* can be explicitly attributed to tree species. Further, less frequently detected tree taxa were *Populus*, *Pinus*, *Quercus*, *Taxus* and *Pseudotsuga*. Charcoal of *Juniperus* mainly occurred on southern exposures, whereas *Betula* was associated with northern exposures. In contrast, the (partly) phreatophytic taxa *Hippophae* and *Salix* showed no prevalent orientation. The distribution of radiocarbon ages on charcoal revealed a discontinuous record of burning events cumulating in the Late Holocene (c. 5700–0 cal BP). For southern Tibet, these results indicated a Late Holocene vegetation change from woodlands to the present desertic pastures. As agrarian economies in southern and south-eastern Tibet date back to c. 3700 and 5700 cal BP, respectively, and the present-day climate is suitable for tree growth up to c. 4600 m a.s.l., we concluded that the Late Holocene loss or thinning out of woodlands had been primarily caused by humans.

1. Introduction

Over the last decade several new studies have dealt with the questions what changes the vegetation cover of central and high Asia underwent during the Holocene and whether the present vegetation is primarily natural or was secondarily established following (past) human impact. Traces of a forest history, as represented by pollen, charcoal and fossil wood, indicate that larger areas of western China, Mongolia and of several Himalayan countries have undergone drastic vegetation changes in the last few millennia (e.g. Beug and Miehe, 1999; Ren, 2000; Shen, 2003; Schlütz and Zech, 2004; Byers, 2005; Feng et al., 2006; Huang et al., 2006; Miehe et al., 2007a; Zhao et al., 2007).

On the Tibetan Plateau, forming the largest alpine area in the world (c. 2.2^{6} km^{2}), treeless vegetation belts in the eastern sector between c. 3000 and 5000 m a.s.l. were often assumed to be natural. Studies dealing with the present-day vegetation and also those on the Late Holocene vegetation history have repeatedly claimed that the region's harsh climatic regime has been the main factor preventing the recent growth of trees and that human impact on vegetational changes has been limited to the recent past (e.g. Ni, 2000; Yu et al., 2001; Ren and Beug, 2002; Luo et al., 2004, 2005; Song et al., 2004). However, there are a growing number of studies which question this view. They point to the current distribution of zonal forest and woodland islands throughout the southern and north-eastern Tibetan Plateau, implying that viable and reproducing tree stands prove that current climatic conditions can support tree

---

[*] Corresponding author. German Academy of Science and Engineering, D-10117 Berlin, Mauerstrasse 79, Germany. Tel.: +49 3312882830; fax: +49 3312881002.
E-mail address: knut.kaiser@gmx.net (K. Kaiser).

0277-3791/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.quascirev.2009.02.016

growth. Furthermore they point to the increasing palaeoecological evidence of larger forests still in the Mid- and at the beginning of the Late Holocene (e.g. Holzner and Kriechbaum, 1998; Frenzel, 2002; Bräuning, 2007; Kaiser et al., 2007; Mosbrugger et al., 2007; Ren, 2007; Schlütz et al., 2007; La Duo, 2008; Miehe et al., 2008a,b; Schlütz and Lehmkuhl, 2009). Consequently, as climatic conditions at the peak of the Holocene desiccation and cooling period (e.g. He et al., 2004; Herzschuh, 2006; Zhao et al., 2007; Zhu et al., 2008) could support tree growth (the tree stands had been established long before the current anthropogenic warming commenced), they claim that climatic changes during the Late Holocene cannot have been the single driving force for a large-scale forest decline. Accordingly forest disappearance or thinning out might be explained by human impact comprising grazing of domestic animals, clearing for the establishment of arable land and settlements, and burning. Furthermore, Kaiser et al. (2006) and La Duo (2008) discuss a combination of climatic effects and anthropic factors, arguing that after an initial climate-driven opening of the vegetation cover by aridification and cooling, human impact increased this process. Nevertheless, as both the evidence for larger Holocene forests and that for early human impact are infrequent, widely-scattered and inadequately discussed so far, the dispute is not settled. With the present paper we want to contribute to this discussion by presenting the so far largest systematically obtained data set of charcoal available from the Plateau in order to provide new regional records of fossil woody taxa. Furthermore, we hope to stimulate additional high-resolution studies by showing the pedoanthracological potential of sites along an 800 km transect.

In general, data on vegetation history can be obtained using several methods/plant remains (e.g. pollen, macro remains, charcoal) and archives (e.g. lake sediments, peat, palaeosols). Evidence and interpretation of past environmental changes substantially depends on how well the archive used represents the area. Palynology usually gains insights into *(sub-) regional* vegetation patterns (e.g. Berglund and Ralska-Jasiewiczowa, 1986; Seppä, 2007; Sugita, 2007). However, because of possible long-distance pollen transport, it remains uncertain whether certain taxa really did grow on the respective sites. In contrast, macroscopic charcoal and fossil wood reliably reflect the *local* growth of woody taxa (e.g. Schoch, 1986; Lynch et al., 2004; Carcaillet, 2007) except for some potentially problematic archives (e.g. fluvial-lacustrine sediments, artificial structures). In comparison to palynological research, which is comparatively well-established on the Plateau, charcoal analysis (anthracology) and fossil wood analysis (xylology) have been rarely performed so far. The charcoal and fossil wood presented in this study were primarily derived from palaeosols and sediments during research on the Late Quaternary geomorphic evolution of the Lhasa area in southern Tibet (Kaiser et al., 2006, 2009, in press-a), and in the context of biogeographical research on the distribution of juniper forests in south-eastern Tibet (Miehe et al., 2008a). They were thus not obtained in a strict anthracological framework. Nevertheless, as the whole material comprised 53 botanical spectra and 55 radiocarbon datings from 46 sampling sites covering a biogeographical transect from the coniferous forests in the east, via the present border of the closed forest area, to forest outposts/relics in the west (Fig. 1, Appendix 1), a palaeoecological appraisal is very much worthwhile.

The overall question to be addressed by this paper is: What can charcoal and fossil wood primarily found in palaeosols and sediments tell us about the Holocene environmental history of southern and south-eastern Tibet? More specifically we wanted to i) describe taxa composition, topographic pattern and dating of (woody) plant fossil assemblages; ii) interpret and discuss the results with respect to the regional environmental history, including fire history, by means of further biogeographical–ecological, palaeobotanical, geoscientific and archaeological evidence; and iii) evaluate the regional pedoanthracological potential.

2. Study areas

We had two main study areas, comprising the Lhasa area in southern Tibet and the deep river gorges of south-eastern Tibet, which as currently classified in the Atlas of Tibet Plateau (1990) belong to two biogeographical zones: open dwarf shrubland pastures and coniferous forests, respectively (Fig. 1, Appendix 1). In the following, mainly information on climate and vegetation/land-use will be given. Further aspects, such as details on geology, geomorphology and pedology, can be taken from e.g. Atlas of Tibet Plateau (1990), Kaiser et al. (2006, 2009, in press-a-b) and Miehe et al. (2008a).

The sites in southern Tibet are located in the Kyichu Valley and its tributaries (Figs. 1 and 2). The deeply incised valley bottoms lie at c. 3500–4200 m a.s.l. surrounded by mountain ridges and peaks up to 5400 m a.s.l. (altitudes sampled: 3500–4600 m a.s.l.). According to climatic data as given in Fig. 1, the present climate on the valley floors at c. 3550–4150 m a.s.l. is characterised by mean annual air temperatures (MAATs) of 2.4–8.5 °C and mean annual precipitations (MAPs) of 361–549 mm a^{-1}, derived from c. 10- to 40-year series of measurements (Domrös and Peng, 1988; Miehe et al., 2001). Rainfall on the higher slopes is considerably higher as exemplary rain-gauge measurements in the Lhasa area showed with an annual rainfall of 485 mm a^{-1} at 3750 m a.s.l. and of 715 mm a^{-1} at 4650 m a.s.l. (Miehe et al., 2003). Penman-Monteith potential evaporation estimates of Lhasa amount to 1328 mm a^{-1} (Thomas and Chen, 2002). There are six to seven months with relatively arid conditions, suggesting a semiarid climate. Domrös and Peng (1988) assign the Lhasa area to a semiarid subtype in the 'Temperate Plateau Zone' of the climate zones of China. The recent snowline is calculated at about 6000 m a.s.l. (Lehmkuhl et al., 2002). Despite the high altitudes, temperatures are suitable for tree growth: between May and September mean monthly temperatures are above 10 °C. The growing season with mean monthly temperatures above 5 °C covers eight months (Miehe et al., 2008a).

The floodplains are characterised by mobile cobbles to sands, sparsely overgrown by grasses, and grazed wetlands with a dense cover of sedges, grasses and herbs, or – if inactive – by irrigated arable land and wood plantations. Grazed remains of the natural phreatophytic woodlands, consisting of tree-forming buckthorn (*Hippophae rhamnoides*), willow (*Salix* spp.) and poplar (*Populus* spp.), are preserved only locally (Figs. 2C and 3A, C, Appendix 2). The valley slopes are exposed to a strong, year-round grazing impact, having a grass-dominated vegetation with low thorny shrubs (e.g. *Sophora moorcroftiana*) and wormwoods (*Artemisia* spp.). In some protected slope positions, shrubs several metres high occur (e.g. *Buddleja* spp., *Cotoneaster* spp.). However, above the valley bottoms large areas are desertic pastures and strongly eroded badlands (Fig. 2A). Above c. 4500 m a.s.l. dense sedge mats of *Kobresia pygmaea* accompanied by cushions prevail. There are several dry-site woodland patches ('forest islands') or single trees of juniper (*Juniperus convallium*, *Juniperus tibetica*) in the study area (Miehe et al., 2008a). The term 'woodland' is used here in the sense of open forests or 'ecosystems that contain widely spaced trees with their crowns not touching' (Hobbs, 2002). The largest woodland is a south-exposed mature *J. tibetica* stand around Reting Monastery, comprising hundreds of trees of 10–15 m high and up to c. 1000 years old; it shows regeneration (c. 4200–4850 m a.s.l.; Miehe et al., 2003, 2008a; Bräuning, 2007; Figs. 2B and 3A, B). Furthermore, small remains of the natural woody vegetation on north-facing slopes consisting of birch (*Betula* spp.), willow (*Salix* spp.) and rhododendron (*Rhododendron* spp.) are preserved in the Kyichu valley and its tributaries at altitudes of c. 4100–4500 m a.s.l. (Figs. 2D and 3A, D).

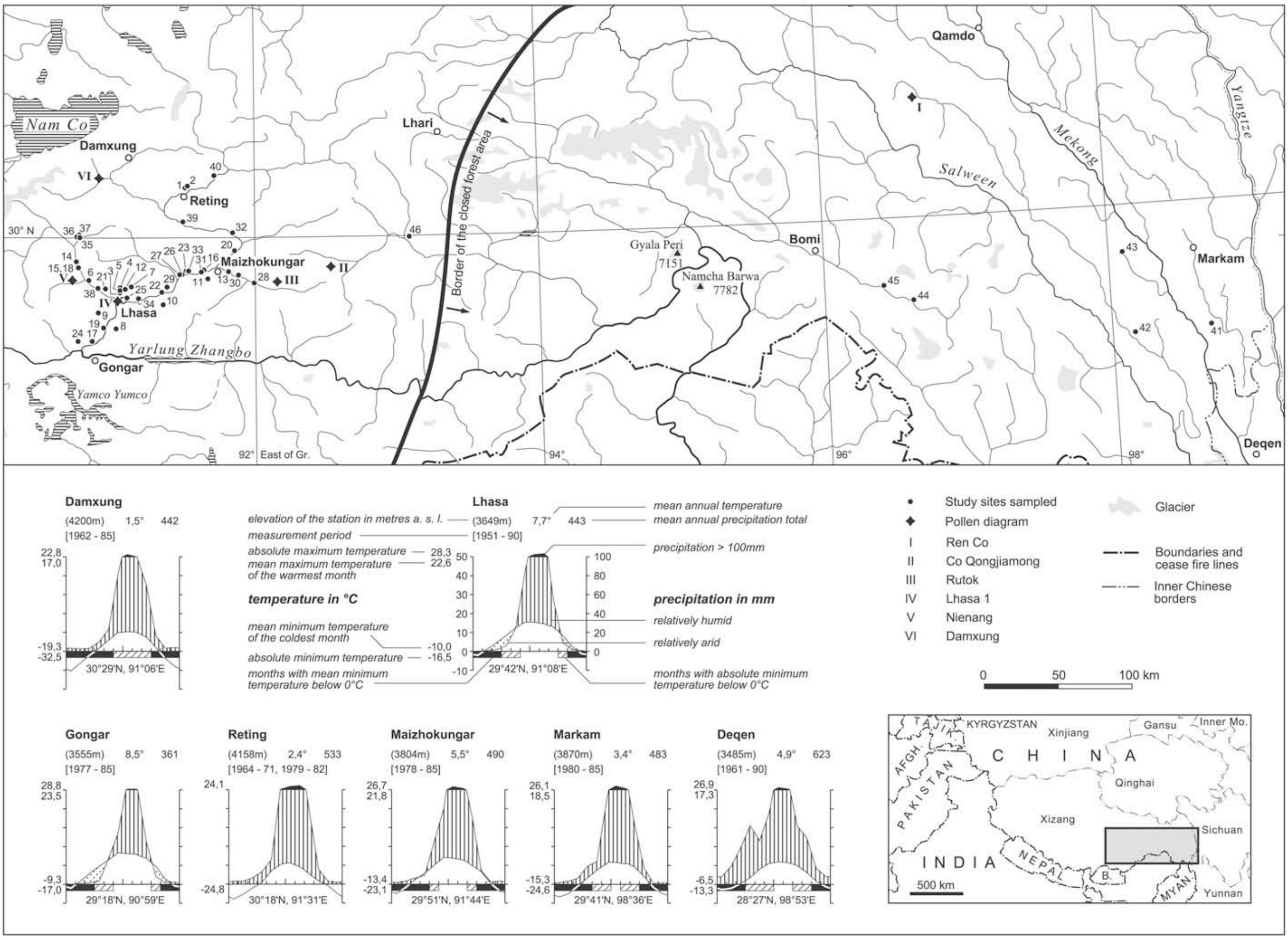


Fig. 1. Location of the sites sampled and of the pollen diagrams discussed (map adapted from Miehe et al., 2001; border of the closed forest area adapted from Atlas of Tibet Plateau, 1990).

The sites in south-eastern Tibet are located within the catchment of the lower Yarlung Zhangbo, Salween and Mekong rivers (Figs. 1 and 2). As the sampling sites are located at c. 3500–4700 m a.s.l., only the meteorological stations Markam (3870 m a.s.l.) and Deqen (3589 m a.s.l.) seem useful for characterising the sites. Their records show MAATs of 3.4 and 4.8 °C and MAPs of 483 and 664 mm a^{-1}, respectively, derived from c. 5- to 26-year series of measurements (Miehe et al., 2001). The area is assigned to a subhumid subtype of the ‘Temperate Plateau Zone’ of China (Domrös and Peng, 1988).

The deeply incised river gorges display a sequence of altitudinal vegetation belts, starting with open dwarf shrublands of the dry river valley bottoms roughly between 2500–3500 m a.s.l. The sequence continues with conifer forests of juniper (*Juniperus* spp.), fir (*Abies* spp.) and spruce (*Picea* spp.) as well as sclerophyllous thickets and forests of oak (*Quercus* spp.) between 3500–4800 m a.s.l. (Fig. 2E–G). The forests are succeeded by thickets (‘krummholz’) and alpine pastures.

3. Material and methods

3.1. General remarks and sampling

In general, (pedo-) anthracological studies have already been performed at several (sub-) alpine sites throughout the world, mostly comprising areally high-resolution palaeoecological case studies (e.g. Tessier et al., 1993; Carcaillet and Thinon, 1996; Tinner et al., 1996; Carcaillet and Brun, 2000; Carnelli et al., 2004; Di Pasquale et al., 2008). In contrast, our data have so far been collected mainly for geomorphical purposes and characterise palaeosols and sediments. Thus we commonly obtained one dated charcoal or fossil wood spectrum per site only, yielding a low areal and temporal resolution. Nevertheless, since the data were derived from a transect extending over c. 800 km west–east distance comprising 46 sampling sites, they represent a valuable cross-section to shed light on the regional vegetation history, particularly of the Lhasa region. Most of both the botanical spectra and datings are published here for the first time, although some have been previously published, comprising 15 spectra and 22 ^{14}C datings (Kaiser, 2004; Kaiser et al., 2006, 2009, in press-a; Appendix 3, Tables 1 and 2).

Field work for the samples presented here took place in between 2003 and 2007. There is a strong disparity in the geographical distribution of the sites sampled, comprising 40 sites in southern Tibet and 6 sites in south-eastern Tibet (Fig. 1). Both natural and anthropogenic exposures with a thickness of 2–13 m were used after preparation of the profiles. In this study, only some selected site properties, such as topographic features (altitude, coordinates and relief), stratigraphical characteristics (sample depth, sediment type, palaeosol and parallel archaeological evidence) and recent vegetation, are documented (Appendix 3). Detailed pedological and sedimentological descriptions of the profiles as well as further analyses (e.g. geochemical and luminescence data) can be obtained from Kaiser et al. (2006, 2009, in

Fig. 2. Photographs of selected sites investigated (an extended caption is available in Appendix 2). (A) Lower Kyichu Valley c. 50 km southwest of Lhasa, southern Tibet (3800 m a.s.l.). The northeast-facing slope in the foreground is covered by an open *Juniperus convallium* stand. (B) Relic *Juniperus tibetica* forest on a south-facing mountain slope (c. 4200–4650 m a.s.l.) in the middle Kyichu Valley west of Reting Monastery, southern Tibet. (C) Valley ground of the Madromachu, southern Tibet (3850 m a.s.l.) covered with *Hippophae rhamnoides* trees up to 5 m high. (D) North-facing slope in the Madromachu Valley, southern Tibet (4150 m a.s.l.), covered by *Betula platyphylla* trees (max. c. 4 m high), *Salix* and *Rhododendron*. (E) Descent from pass west of Garthok, south-eastern Tibet (4250 m a.s.l.). Forest on the southwest-facing slope is mainly made up of *Picea* with *Quercus* and *Juniperus*. (F) View from sampling site LO-04-H6 at the outflow of Rawu Lake, south-eastern Tibet (3950 m a.s.l). Forest on the northwest-facing slope is mainly made up of *Picea purpurea*. (G) Single *Juniperus* trees on a southwest-facing slope in south-eastern Tibet (4130 m a.s.l.). The north-facing slope in the background is covered by *Picea* forest. (H) Large piece of *Juniperus* charcoal (max. 13 cm in length) in profile SHE 1, southern Tibet (3700 m a.s.l.), dating to 2624 ± 103 cal BP. (I) Large pieces of *Populus* (max. 30 cm in length) in profile CHU 5, southern Tibet (3770 m a.s.l.), dating to 3586 ± 59 cal BP. (J) Profile FAN 1, southern Tibet (3720 m a.s.l.), showing a palaeosol (red dot) with fluvial sand below and above. Charcoal (e.g. *Juniperus*) extracted from the palaeosol dates 2511 ± 129 cal BP. (K) Profile STA 1, southern Tibet (3660 m a.s.l.), showing a palaeosol (red dot) with aeolian sand below and above. Charcoal (*Juniperus*) extracted from the palaeosol dates 2817 ± 34 cal BP. (L) Profile CHS 1, southern Tibet (4040 m a.s.l.), showing two palaeosols (red and blue dot) developed from fluvial sand and silt. Charcoal (e.g. *Hippophae*) extracted from the palaeosol dates 6603 ± 59 cal BP (red dot) and 6808 ± 62 cal BP (blue dot). (M) Profile CHU 5, southern Tibet (3770 m a.s.l.), showing a peat layer (red dot) below fluvial and colluvial silt. Fossil wood (*Populus*) extracted from the peat dates 3586 ± 59 cal BP.

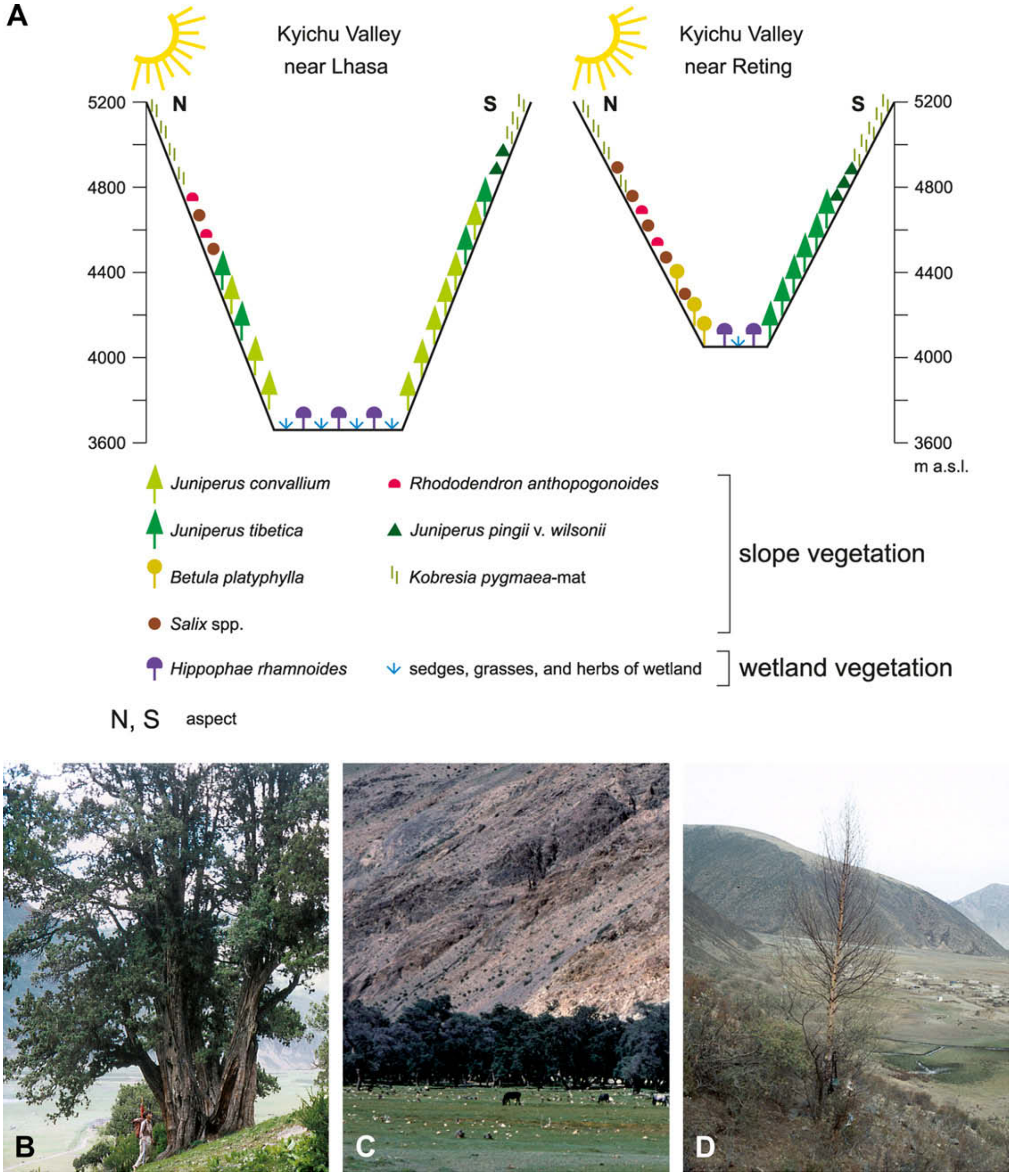


Fig. 3. Vegetation characteristics of valleys in southern Tibet. (A) Natural altitudinal vegetation belts in the Kyichu Valley derived from isolated tree stands and vegetation records (adapted from Miehe et al., 2008a). (B) *Juniperus tibetica* tree (c. 13 m high, c. 2.3 m breast height diameter, DBH) on a south-facing lower slope at Reting site (4200 m a.s.l., 30°18′N, 91°30′E). (C) *Hippophae rhamnoides* trees (c. 10 m high, c. 0.5 m DBH) in the upper Arun Valley (c. 4000 m a.s.l., 28°20′N, 87°12′E). (D) *Betula platyphylla* tree (c. 8 m high, c. 0.2 m DBH) on a north-facing lower slope near Reting site (4100 m a.s.l., 30°17′N, 91°30′E).

press-a). The charcoal samples ($n = 50$) and fossil wood samples ($n = 3$) were picked out macroscopically, using knives and tweezers, from the whole layer sampled (e.g. palaeosol). After air-drying and sieving, c. 50–200 particles per (charcoal-) sample comprising a bulk volume of c. 5–20 cm^3 were stored in plastic tubes.

3.2. Botanical determination

The microscopic examination of charcoal and fossil wood samples was carried out on fracture surfaces and microsections, respectively, on the three fundamental planes (transverse, longitudinal–radial and longitudinal–tangential; Appendices 4 and 5) under the stereomicroscope Olympus BX-60 (magnifications for botanical determination: 50–500×). Identification was performed by means of comparison with preparations from living material or from digital photographs of recent wood species (Hoadley, 1990; Schweingruber, 1990; Schoch et al., 2004).

The fossil taxa determined were arranged into ‘ecological groups’ showing properties (growth form, exposure) of their probable *present-day* equivalents in southern and south-eastern Tibet (Table 1). The assignation of certain taxa to growth forms is discussed in detail in chapter ‘5.1. Assignation of growth forms’.

3.3. Radiocarbon dating

Radiocarbon (^{14}C) dating was performed on a total of 55 samples in the Erlangen Radiocarbon Laboratory by accelerator mass spectroscopy (AMS; Table 2). The majority of ages were obtained from

Table 1
Fossil plant taxa determined in southern and south-eastern Tibet and their arrangement in ecological groups.

Ecological group	Taxon determined	English name	Growth form[a]	Remark
Upland tree species I (southern Tibet)	*Juniperus sp.*	juniper	Tr	probably *J. tibetica*, mostly south-facing growth
	Betula sp.	birch	Tr	probably *B. platyphylla*, mostly north-facing growth
	Salix sp.	willow	Tr	partly north-facing growth (partly phreatophytic)
	Pinus sp.	pine	Tr	imported timber?
Upland tree species II (south-eastern Tibet)	*Juniperus sp.*	juniper	Tr	mostly south-facing growth
	Betula sp.	birch	Tr	mostly north-facing growth
	Taxus sp.	yew	Tr	mostly north-facing growth
	Pseudotsuga sp.	Douglas-fir	Tr	mostly north-facing growth
	Pinus sp.	pine	Tr	mostly south-facing growth
	Quercus sp.	oak	Tr	mostly south-facing growth
	Salix sp.	willow	Tr	partly north-facing growth, partly phreatophytic
Wetland tree and shrub species	*Salix sp.*	willow	Tr	partly phreatophytic (partly north-facing growth)
	Populus sp.	aspen	Tr	–
	Hippophae sp.	buckthorn	Tr	probably *H. rhamnoides*
	Tamaricaceae	tamarisk family	Sh	probably *Myricaria* spp.
Shrub species of the forest understorey and above treeline	*Buddleja sp.*	butterfly bush	Sh	mostly south-facing growth
	Lonicera sp.	honeysuckle	Sh	–
	Sophora sp.	necklacepod	Sh	probably *S. moorcroftiana*, mostly south-facing growth
	Caragana sp.	pea shrub	Sh	–
	Clematis sp.	clematis	He	–
	cf. *Rhododendron*	rhododendron	Sh	mostly north-facing growth
	cf. *Vaccinium*	blueberry	Ds	mostly north-facing growth
	Rosaceae/Maloideae	stone fruit family	Sh	–
	Ericaceae	heath family	Ds	–
	Spiraea sp.	spirea	Sh	–
Cultivated species	Poaceae	grasses	Gr	probably barley (*Hordeum vulgare*)
Further species	*Rosa sp.*	rose	Sh	–
	Fabaceae	pea family	He	–
	cf. Liliaceae	lily family	He	–

[a] Abbreviations: Tr = tree, Sh = shrub, Ds = dwarf-shrub, Gr = grass, He = herb.

charcoal ($n = 50$) and fossil wood ($n = 3$). Most analyses ($n = 44$) were performed on genus- or, infrequently, family-specific plant material. In two cases, charcoal spectra could be dated only indirectly using bone and bulk-soil matter (samples QUG 1, SAI 1b), respectively, bearing a clear risk of data rejuvenation by infiltration with younger carbon. Treatment of the samples followed the standard methods of the Erlangen Radiocarbon Laboratory (Scharf et al., 2007). All ^{14}C ages presented were calibrated (cal BP-values, 1 sigma-calibration) using the program 'CalPal-2007' (Weninger et al., 2007).

4. Results

4.1. Origin and taphonomic aspects of the samples

Most samples derived from palaeosols ($n = 34$). Further samples originated from fluvial-lacustrine ($n = 6$), aeolian ($n = 6$) and colluvial sediments ($n = 5$). Four samples (RET 1, RET 8, LSW 3, GHU 1) were taken from artificial structures (burial mounds, field terraces), partly in combination with palaeosols. The sampling depth varied from 20 to 1000 cm (mean = 281 cm), whereas the thickness of the layers sampled ranged from 2 to 200 cm (mean = 24 cm). In six cases different levels per profile were sampled (Appendix 3). Thus, in general, the samples provided a proper proxy for the local species composition with high spatial precision in contrast to low temporal resolution.

Most sites investigated were footslopes ($n = 22$), followed by alluvial fans ($n = 7$), floodplains of rivers and streams ($n = 6$), middle slopes ($n = 5$), river terraces ($n = 4$), and lower slopes ($n = 2$). The altitudinal range of the sites sampled was c. 3500 to 4700 m a.s.l. More detailed relationships between relief characteristics and the botanical spectra determined will be addressed in chapter '4.2. Botanical determination'.

According to the lack of properties for redistribution (e.g. layering, soil clasts, enrichment of coarse particles), the palaeosols sampled can be widely regarded as *in-situ* formations (see Kaiser et al., 2006, 2009, in press-a) implying a more or less (para-) *autochthonous* position of the charcoal and fossil wood particles. The palaeosols represented both dry-site conditions (Phaeozems, Kastanozems, Calcaric Cambisols, Arenosols and Regosols) and wet-site conditions (Gleysols, Histosols). Taking the mountainous relief into account, a potential short-distance input of the charcoal particles from the immediate surroundings – e.g. transported by slope runoff – cannot be precluded. In contrast, the samples from fluvial-lacustrine and colluvial sediments must as a rule be considered to be in *allochthonous* position, potentially originating from sources a few hundred metres to many kilometres away. Most of the palaeosols and sediments sampled had strongly dispersed and fragmented charcoal only.

The combined appearance of artificial (archaeological) objects and charcoal is a matter of particular interest comprising 10 spectra (Appendix 3). There were combinations both with human occupation layers (profiles DRE 2, DRE 14, GAR 1, QUG 1, BRI 1, FAN 1, GUZ 1), containing e.g. pot shards, lithic artefacts and bones, and with anthropogenic constructions (burial mounds, field terraces; profiles RET 1, RET 8, LSW 3, GHU 1).

The charcoal particles sampled were normally of 1–5 mm in length. Larger objects up to 2 cm in length were scarcely found – mostly in connection with human occupation layers. The largest charred object found is a tree stem or branch of 13 cm in length and 5 cm in diameter (sample SHE 1a; Fig. 2H). In contrast, fossil wood normally attains larger dimensions with a maximum length and diameter of 30 cm and 8 cm, respectively (sample CHU 5; Fig. 2I).

Table 2
Radiocarbon datings from southern and south-eastern Tibet.

Code	Sample	Altitude [m a.s.l.]	Northing	Easting	Depth [cm]	Material dated[a]	Lab. No.	δ^{13}C [‰]	Age uncalibrated [BP]	Age calibrated [cal BP]	Reference
Sites in southern Tibet											
5	DRE 14	3846	29°40′42.8″	91°02′58.0″	200	*Juniperus* charcoal	Erl-6778	−21.6	−13 ± 42	–	Kaiser et al., 2006
3	DRE 2	3654	29°40′04.6″	91°02′57.4″	145–150	*Juniperus* charcoal	Erl-6776	−21.6	203 ± 41	175 ± 111	Kaiser et al., 2006
2a	RET 8a	4224	30°19′07.3″	91°31′32.8″	20–50	*Juniperus* charcoal	Erl-6780	−22.2	243 ± 55	280 ± 123	Kaiser, 2004
40a	GUZ 1a	4080	30°22′55.6″	91°42′52.3″	90–95	*Juniperus* charcoal	Erl-11518	−19.9	246 ± 48	284 ± 120	this study
2b	RET 8b	4224	30°19′07.3″	91°31′32.8″	110–140	*Rosa* charcoal	Erl-6781	−25.2	891 ± 46	826 ± 64	Kaiser, 2004
29	BON 1b	3684	29°41′57.8″	91°22′47.6″	195–285	*Salix* charcoal	Erl-10943	−25.8	903 ± 46	833 ± 61	this study
1	RET 1	4200	30°18′28.7″	91°30′33.6″	80–114	*Juniperus* charcoal	Erl-6779	−22.0	1197 ± 45	1131 ± 59	Kaiser, 2004
35a	CNG 4a	4043	30°00′44.9″	90°45′37.0″	200–250	TS charcoal	Erl-11512	−21.0	1480 ± 51	1383 ± 50	this study
17	BRI 1	3566	29°21′58.1″	90°51′17.8″	115–150	*Sophora* charcoal	Erl-10938	−24.8	1865 ± 51	1803 ± 61	Kaiser et al., 2009
12a	LSW 3	3684	29°41′10.6″	91°05′10.9″	380	*Hippophae* wood	Erl-11522	−25.2	1869 ± 37	1806 ± 53	this study
27	ZAN 2b	3670	29°46′31.0″	91°28′24.3″	270–280	*Hippophae* charcoal	Erl-10952	−24.9	2055 ± 50	2030 ± 68	this study
31	MOG 2e	3777	29°47′40.4″	91°37′33.5″	390–410	*Betula* charcoal	Erl-10942	−25.5	2159 ± 51	2183 ± 99	Kaiser et al., 2009
33	GHU 2	3755	29°47′50.9″	91°31′41.1″	200–210	TS charcoal	Erl-11510	−21.1	2162 ± 52	2185 ± 98	this study
4	DRE 9	4583	29°41′42.1″	91°03′01.8″	30–57	*Juniperus* charcoal	Erl-6777	−21.0	2194 ± 41	2227 ± 68	Kaiser et al., 2006
23	GHU 1	3722	29°47′47.1″	91°31′02.5″	150–170	*Juniperus* charcoal	Erl-10949	−21.1	2296 ± 50	2276 ± 72	this study
40c	GUZ 1c	4080	30°22′55.6″	91°42′52.3″	200–202	*Hippophae* charcoal	Erl-11520	−22.9	2362 ± 55	2447 ± 94	this study
38	FAN 1	3721	29°41′32.3″	90°53′40.5″	370–390	TS charcoal	Erl-11516	−24.9	2399 ± 51	2511 ± 129	this study
40b	GUZ 1b	4080	30°22′55.6″	91°42′52.3″	160–180	TS charcoal	Erl-11519	−22.4	2409 ± 53	2519 ± 131	this study
12b	LSW 2	3684	29°41′10.6″	91°05′10.9″	250–265	DW charcoal	Erl-10944	−25.0	2500 ± 49	2590 ± 101	this study
34	SHE 1a	3699	29°37′43.6″	91°10′45.9″	210–230	*Juniperus* charcoal	Erl-11511	−22.3	2548 ± 57	2624 ± 103	this study
25	STA 1	3658	29°37′59.4″	91°05′52.0″	135–150	*Juniperus* charcoal	Erl-10940	−20.9	2713 ± 38	2817 ± 34	Kaiser et al., 2009
22	DAR 1	3662	29°40′01.7″	91°20′35.6″	300–325	*Hippophae* charcoal	Erl-10948	−25.5	2970 ± 52	3152 ± 83	Kaiser et al., 2009
7	QUG 1	3679	29°42′03.9″	91°07′42.2″	135–170	animal bone	Erl-6783	−14.8	3053 ± 45	3275 ± 58	Kaiser et al., 2006
39	NAN 1b	3997	30°05′57.6″	91°29′35.2″	330–340	*Hippophae* charcoal	Erl-11517	−23.8	3073 ± 52	3289 ± 61	this study
18	SAI 2	3804	29°49′03.6″	90°45′25.0″	200–400	*Juniperus* charcoal	Erl-11521	−18.1	3075 ± 39	3300 ± 47	this study
9	CHU 5	3770	29°32′27.7″	90°53′49.8″	385–438	*Populus* wood	Erl-10117	−27.1	3353 ± 40	3586 ± 59	this study
30	MAN 1b	3960	29°46′13.5″	91°52′57.0″	110–120	*Betula* charcoal	Erl-10945	−24.4	3485 ± 51	3766 ± 63	this study
6	GAR 1	3800	29°44′25.5″	90°49′49.3″	200–208	*Juniperus* charcoal	Erl-6782	−20.5	3668 ± 57	4005 ± 79	Kaiser et al., 2006
20	DON 2	4027	29°55′13.4″	91°51′22.5″	70–100	*Betula* charcoal	Erl-10950	−24.7	3872 ± 55	4298 ± 89	this study
32a	TAG 1a	3920	30°01′49.2″	91°50′35.5″	350–365	TS charcoal	Erl-11508	−23.4	4088 ± 60	4645 ± 125	this study
28	BAL 1b	4085	29°43′16.7″	91°59′34.4″	430–470	*Betula* charcoal	Erl-10951	−25.5	4804 ± 58	5535 ± 58	this study
8a	CHS 1a	4044	29°26′36.8″	91°01′17.7″	255–280	TS charcoal	Erl-10115	−24.5	5803 ± 48	6603 ± 59	Kaiser et al., in press-a
8b	CHS 1b	4044	29°26′36.8″	91°01′17.7″	450–485	*Hippophae* wood	Erl-10116	−26.7	5964 ± 49	6808 ± 62	Kaiser et al., in press-a
32b	TAG 1b	3920	30°01′49.2″	91°50′35.5″	390–400	*Juniperus* charcoal	Erl-11509	−23.2	6402 ± 63	7341 ± 60	this study
10	DAV 2	3909	29°35′27.8″	91°21′10.8″	500–535	*Buddleja* charcoal	Erl-10118	−26.3	6586 ± 55	7499 ± 48	this study
26	ZAN 1b	3705	29°46′33.9″	91°28′59.4″	220–250	*Hippophae* charcoal	Erl-10941	−23.0	6925 ± 69	7771 ± 70	Kaiser et al., in press-a
24	QUX 2	3536	29°21′57.3″	90°45′20.2″	325–330	DW charcoal	Erl-10946	−24.8	6943 ± 65	7784 ± 70	Kaiser et al., 2009
21	DYU 1	3704	29°41′19.9″	90°56′51.9″	590–600	*Sophora* charcoal	Erl-10947	−25.1	7375 ± 67	8196 ± 100	Kaiser et al., in press-a
35b	CNG 4b	4043	30°00′44.9″	90°45′37.0″	300–330	*Hippophae* charcoal	Erl-11513	−22.5	7666 ± 71	8477 ± 60	this study
19	NYM 1	3511	29°26′55.1″	90°56′03.6″	240–255	*Sophora* charcoal	Erl-10939	−24.8	7827 ± 70	8654 ± 105	Kaiser et al., 2009
37	CNG 1	4256	30°00′25.0″	90°45′44.1″	120–130	Liliaceae charcoal	Erl-11515	−23.5	8183 ± 65	9155 ± 96	this study
15	SAI 1b	3804	29°49′03.6″	90°45′25.0″	460–470	bulk-soil matter	Erl-10122	−19.8	8233 ± 68	9216 ± 109	Kaiser et al., 2009
36	CNG 2	4074	30°00′25.7″	90°45′11.2″	100–113	*Hippophae* charcoal	Erl-11514	−24.7	12106 ± 607	14453 ± 889	this study
14	MAR 1	3890	29°51′18.1″	90°44′30.4″	1000	*Caragana* charcoal	Erl-10121	−22.2	30050 ± 654	34247 ± 619	this study
16	MOG 1c	3778	29°47′57.0″	91°38′06.2″	860–885	*Lonicera* charcoal	Erl-10123	−25.5	36273 ± 2327	40305 ± 2312	Kaiser et al., 2009
11	GYA 3	3840	29°44′54.6″	91°40′02.5″	380–395	Rosa./Malo. charcoal	Erl-10119	−24.4	44235 ± 3388	48681 ± 3786	Kaiser et al., 2009
13	MOG 4	3894	29°47′30.0″	91°48′45.2″	720–760	TS charcoal	Erl-10120	−22.6	45682 ± 5438	51024 ± 6148	Kaiser et al., 2009
Sites in south-eastern Tibet											
45	LO-04-H7	3500	29°35′16.6	96°24′50.8	180	*Pinus* charcoal	Erl-10135	−22.4	597 ± 36	601 ± 38	this study
43	LO-04-H5	4670	29°42′31.6	98°05′58.5	155	*Juniperus* charcoal	Erl-10132	−21.5	1200 ± 37	1130 ± 49	this study
46	LO-04-H9	3568	29°59′38.8	93°05′20.6	160	*Quercus* charcoal	Erl-10136	−25.2	2445 ± 40	2535 ± 126	this study
44a	LO-04-H6-J	3950	29°29′28.0	96°37′06.3	120	*Juniperus* charcoal	Erl-10133	−24.7	4498 ± 46	5165 ± 97	this study
44b	LO-04-H6-Ps	3950	29°29′28.0	96°37′06.3	120	*Pseudotsuga* charcoal	Erl-10134	−24.1	4632 ± 46	5385 ± 61	this study
41b	LO-04-H2-T	4020	29°13′58.4″	98°41′22.8″	60	*Taxus* charcoal	Erl-10130	−24.6	6768 ± 56	7627 ± 37	this study
41a	LO-04-H2-J	4020	29°13′58.4″	98°41′22.8″	60	*Juniperus* charcoal	Erl-10129	−24.4	6805 ± 54	7648 ± 38	this study
42	LO-04-H3	3600	29°12′45.3″	98°09′18.2″	240	*Juniperus* charcoal	Erl-10131	−23.9	9456 ± 66	10792 ± 173	this study

[a] Abbreviations: TS = total spectrum, DW = deciduous wood.

4.2. Botanical determination

In general, identification to the species level was unfeasible due to the lack of a regional wood anatomy reference database. Furthermore, the charcoal particles available were mostly relatively small (often <1 mm). Thus it was hardly possible to consider the variability of structures within a taxon (e.g. size, number and distribution of pores and parenchymacells, width of rays) for delineation down to a species level. Additionally, the small fragments did not allow the origin of the wood (from stem, branch, twig or root) to be distinguished. Often only late wood was preserved. Thus important features were not clearly recognisable (e.g. shape of ray cell pits, character of ray tracheid walls) preventing the determination of species.

Therefore most of the charcoal and fossil wood samples were determined on a genus level, with some exceptions that could only be determined on even higher taxonomic levels (Fig. 4, Appendix 3). Some taxa may represent either trees or shrubs (*Juniperus*, *Hippophae*, *Salix*). Nevertheless, an assignation to certain growth forms ('species') is possible considering both the current regional vegetation cover and the regional environmental history (see chapter '5.1. Assignation of growth forms').

The number of particles determined per sample (spectrum) amounted to 2–129 for charcoal (mean = 39.3, $n = 50$ samples) and to 1–7 for fossil wood ($n = 3$ samples; Appendix 3). In general, most of the taxa determined (Appendix 3) represent shrubs/dwarf-shrubs ($n = 13$), followed by trees ($n = 9$) and herbs/grasses ($n = 4$). With respect to the frequency of the trees recorded, the genera *Hippophae* and *Juniperus* prevail (Fig. 4). The tree genera *Quercus*, *Taxus* and *Pseudotsuga* were restricted to south-eastern Tibet only, corresponding to their current distribution area (Atlas of Tibet Plateau, 1990). A relationship between tree taxa and general relief position (valley floor, alluvial fan, different slope sections) cannot be derived due to the preferential sampling of footslope and valley floor positions. However, a relationship between exposure and taxon (Fig. 5) is apparent among *Juniperus*, which was mainly recorded on or below south-facing slopes, and *Betula*, which was mainly associated with a general north exposure. In contrast, the (partly) phreatophytic *Hippophae* and *Salix* show no clear prevalent orientation.

Several attempts were made to extract pollen from the palaeosols, aiming at a further palaeobotanical proxy in addition to the charcoal record (F. Schlütz, Göttingen, pers. comm.). However, with a few exceptions of wet-site palaeosols, most of the buried soil horizons did not contain pollen, which was probably caused by the long-term corrosion of plant remains in the well-aerated soil (Havinga, 1984).

4.3. Dating

There is a strong disparity of prevalently Holocene ages ($n = 50$) in comparison to a few Pleistocene ages ($n = 5$; Fig. 6A). The Holocene (charcoal) data set on its own also shows disparity, with a dominance of Late Holocene ages (c. 5700–0 cal BP, $n = 24$), followed by Mid-Holocene ages (c. 8900–5700 cal BP, $n = 9$) and Early Holocene ages (c. 11,500–8900 cal BP, $n = 2$; Fig. 6B).

Radiocarbon dating of charcoal raises no difficulties provided that there is no contamination by younger humic substances carried by groundwater or stagnant water (Alon et al., 2002). The majority of datings ($n = 36$) represent a single radiocarbon age per profile (Table 2). Consequently, in most cases a given charcoal or fossil wood age can only be assumed to be reliable, i.e. synchronous with the formation age of its bearing palaeosol or sediment. But for some profiles ($n = 19$) either two or more ^{14}C dates are available or independent age control is available from further geochronological (OSL, IRSL) and archaeological data (Kaiser et al., 2006, 2009, in press-a). This exemplary control shows that the *Holocene* ages, except sample QUX 2 (a Mid-Holocene charred root in Pleistocene sediments), can be widely regarded as reliable. Two of the five *Pleistocene* ages, however, are considered to be unreliable (samples MOG 1, MOG 4). Considering the parallel OSL ages available (Kaiser et al., 2009) and keeping in mind the limits of the radiocarbon dating technique (e.g. Geyh, 2005), these ^{14}C age estimates should be regarded as minimum ages only. They may have been contaminated with younger carbon by infiltration of humic substances in palaeosols and are at the upper age limit of the technique.

Most datings were performed on one selected taxon per botanical spectrum only (= *direct* dating). Charcoal layers have been shown to accumulate charcoals for up to a few hundred years depending on the duration of soil and sediment genesis. For the purpose of this study age classes of a few hundred years provided sufficient resolution. Therefore the single datings were considered representative for all specimen of that particular spectrum (= indirect or *assigned* dating; Fig. 7), which has to be verified in the future for a wider spectrum of samples. This assumption was checked in two cases where two taxa per spectrum were dated. These datings confirmed the assumption with LO-04-H2 dates of 7648 ± 38 cal BP (*Juniperus*) and 7627 ± 37 cal BP (*Taxus*), and LO-04-H6 dates of 5165 ± 97 cal BP (*Juniperus*) and 5385 ± 61 cal BP (*Pseudotsuga*). The totals of direct and assigned datings were 24 for *Hippophae*, 20 for *Juniperus*, 13 for *Salix*, 9 for *Betula* and 1–3 for *Populus*, *Pinus*, *Quercus*, *Taxus* and *Pseudotsuga*.

According to these fossil records *Hippophae* was present both in the Pleistocene and Holocene, whereas *Juniperus* and *Betula* are recorded from the (entire) Holocene. The 13 *Salix* records are all

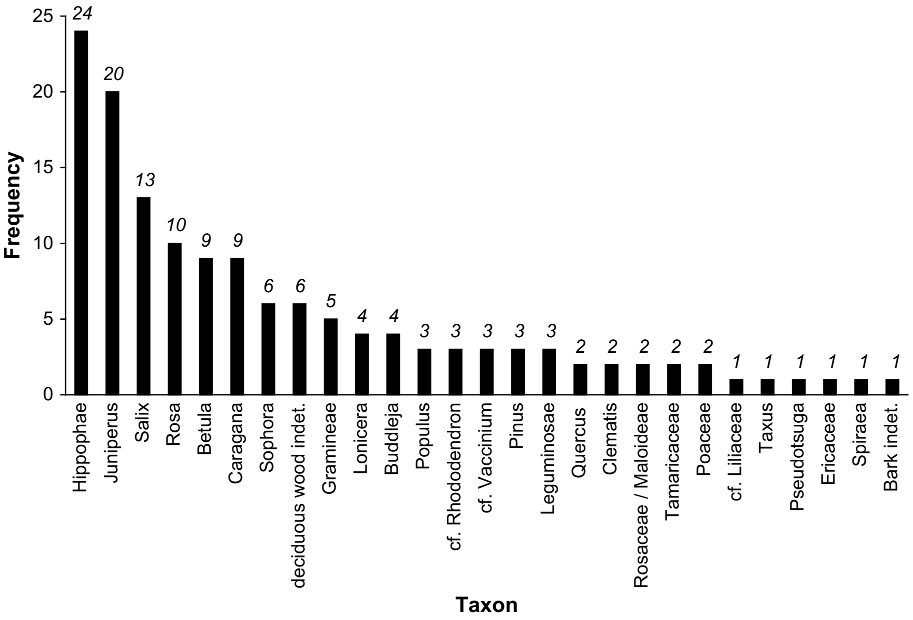


Fig. 4. Frequency distribution of the taxa recorded. The term 'Poaceae' comprises charred Poaceae seeds (a cereal, probably barley).

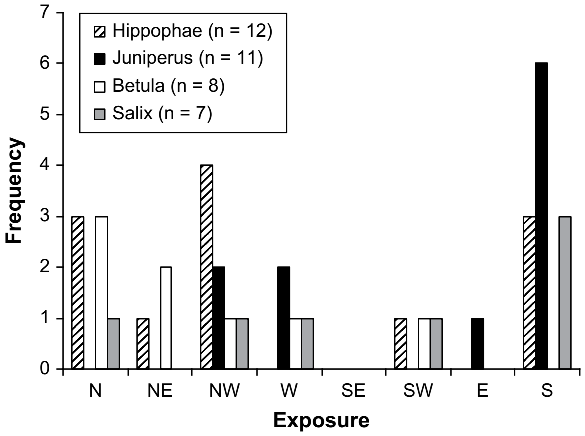


Fig. 5. Exposure of sampling sites (slopes) for selected taxa recorded.

attributed to the Late Holocene. In absolute numbers, there is a clear cluster of Late Holocene ages.

5. Discussion

5.1. Assignation of growth forms

As the *Juniperus* species cannot be distinguished from each other on the basis of their wood anatomy, so far the assignment of charcoal and fossil wood must consider both shrubby and treelike growth forms. Juniper trees (e.g. *J. tibetica*; Fig. 3B) are found up to an altitude of c. 4900 m a.s.l., whereas juniper shrubs (*Juniperus pingii* v. *wilsonii*) form thickets ('krummholz') in the treeline ecotone between c. 4200 and 5300 m a.s.l. (Farjon, 2005; Miehe et al., 2007b, 2008a). According to palaeoclimatic results for the Tibetan Plateau (e.g. He et al., 2004; Herzschuh, 2006; Zhao et al., 2007; Zhu et al., 2008), a Mid- to Late Holocene *lower* treeline than at present, which would affect the altitudinal distribution of *Juniperus* species, can most probably be precluded. Thus all of our records of *Juniperus* originate from sites clearly below the treeline. Furthermore, most of our *Juniperus* records (15 of 20) are from sites below the altitudinal distribution range of the shrubby junipers. Of the remaining five sites, three are still forested even today. It thus seems reasonable to assign the juniper charcoals to treelike growth forms.

The same line of argument can be employed regarding *Hippophae*. This taxon comprises tree species and dwarf-shrub species in the region as well: *Hippophae rhamnoides* occurs on river floodplains and further water-surplus sites (stream valleys, lower slopes, basin floors) forming trees up to 10 m high (Wu, 1983–1987; Fig. 3C). In contrast, the dwarf-shrub species *Hippophae tibetana*, which is maximally 0.5 m high, grows along streams in alpine altitudes (>c. 4200 m a.s.l.). Of the 23 *Hippophae* records in this study only one fitted the known distribution range of *H. tibetana* with an elevation higher than 4200 m a.s.l.

Finally several *Salix* species below c. 4500 m a.s.l. (c. 70 species in southern and south-eastern Tibet; Wu, 1983–1987) and all *Betula* species (prevailing *Betula platyphylla*; Miehe et al., 2008a; Fig. 3D) occurring in the study areas are trees.

In conclusion, it seems justified to generalise by referring to trees when discussing *Juniperus*, *Salix*, *Hippophae* and *Betula* remains in this study.

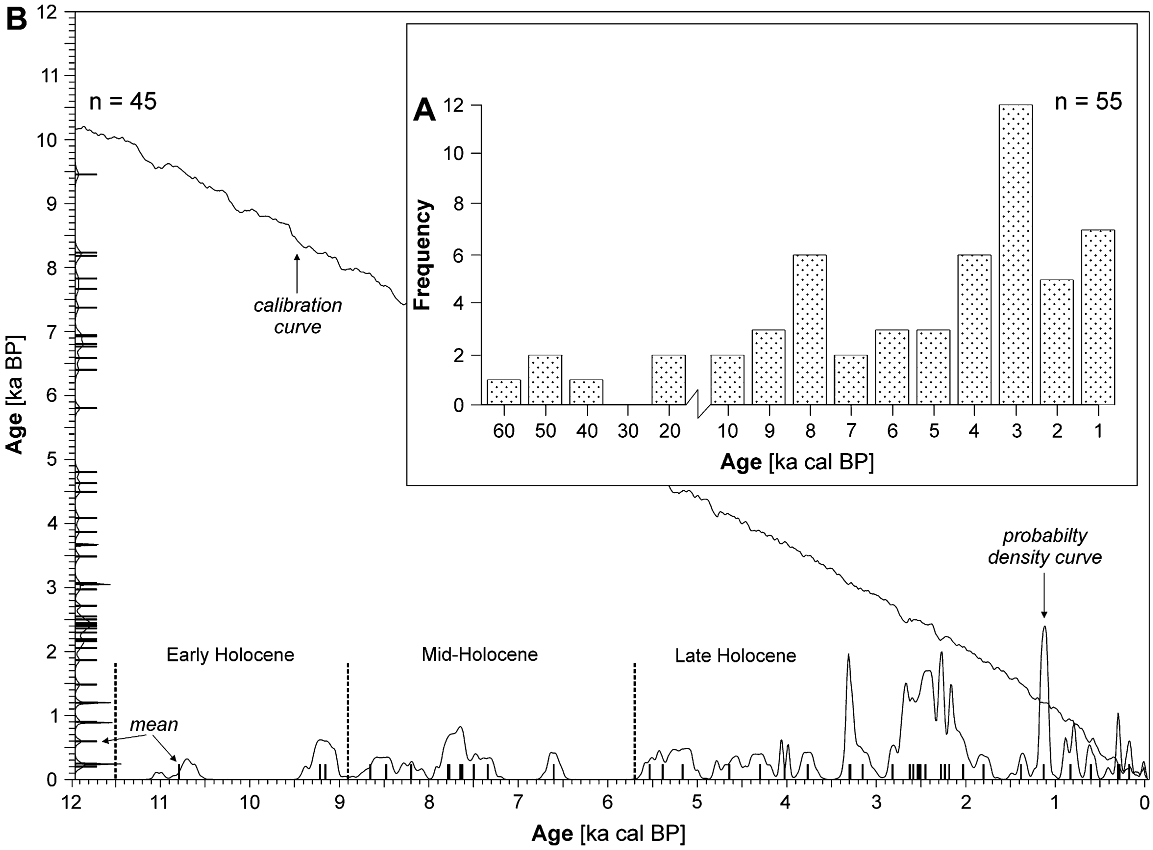


Fig. 6. Frequency and temporal distribution of the radiocarbon ages yielded. (A) Frequency of the age means in terms of millennia for all data yielded. (B) Calibration of the Holocene ages on charcoal.

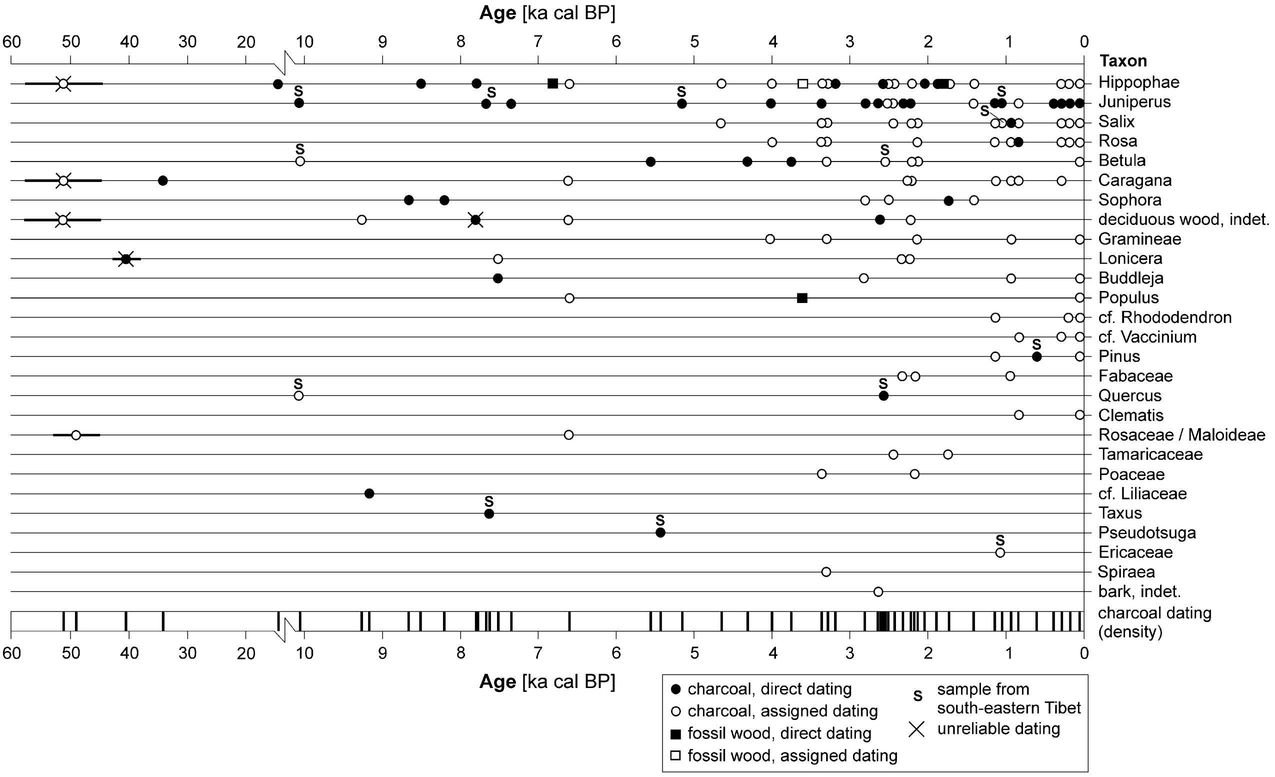


Fig. 7. Age spectra of the taxa determined. Most datings were performed on one selected taxon per botanical spectrum only (= *direct* dating) and were considered representative of all specimens of that particular spectrum (= indirect or *assigned* dating).

5.2. Past woodland composition and distribution

Local ('isolated') *present-day* occurrences of trees covering slopes (*Juniperus*, *Betula*) and valley grounds (*Hippophae*, *Salix* and *Populus*) played a significant role for both the assignation of growth forms in our data set (= identification of possible species) and for the reconstruction of past vegetation in the study area. A correlation with tree stands and rainfall data of some climate stations reveals that the drought line of *Juniperus* trees in southern Tibet is c. 200–250 mm a^{-1} (Miehe et al., 2008a). Accordingly, all of our study sites (c. 3500–4700 m a.s.l.) belong to an area of *potential* present-day tree growth.

On the one hand our study indicated *former* growth of tree taxa at various sites in the treeless desert pastures of southern Tibet. Deduced from the properties of present-day tree occurrences, and corroborated by the charcoal record presented, three *fossil* woodland types can be designated: (1) *Juniperus* woodland on (in general) south-facing slopes, (2) *Betula*(-*Salix*) woodland on north-facing slopes, and (3) *Hippophae*-dominated woodland with *Salix* and *Populus* on water-surplus sites, such as valley floors, basins and footslopes. The temporal distribution of selected tree taxa shows a predominant Late Holocene record in southern Tibet (Fig. 8). Further fossil woody taxa, such as *Rosa*, *Caragana*, *Sophora* and *Buddleja*, which also presently occur in the pastures, may have originally represented shrub species of the forest understory and/or may represent elements of a secondary vegetation. In most cases their presence in the charcoal spectra was paralleled by the presence of tree taxa (e.g. *Juniperus*).

On the other hand the charcoal record from sites in *south-eastern* Tibet partly shows former tree growth (*Juniperus*, *Taxus*) at sites with present-day *Kobresia* sedge mats (c. 4000–4700 m a.s.l.), and partly reveals the continuous Holocene presence of forests (mixed deciduous and coniferous forests, c. 3500–4000 m a.s.l.) by the record of accordant tree taxa (*Quercus*, *Betula*, *Juniperus*, *Pseudotsuga* and *Pinus*).

More highly resolved evidence on the Holocene vegetation history of the study areas is available from six pollen diagrams virtually forming a c. 800-km-long east–west transect from the closed forest area in south-eastern Tibet to the alpine pastures in southern central Tibet (Fig. 1, Table 3). The sediments investigated are predominantly lacustrine deposits and peats allowing reliable reflections of the Holocene vegetation history. However, three of the diagrams do not contain Late Holocene sediment sections (hiatuses), suggesting either that none were deposited or that they were subsequently eroded. The sites are currently treeless except Damxung, where a small stand of *J. tibetica* (up to 4 m high) occurs.

To sum up, five of the six pollen diagrams presented show a Late Holocene replacement of tree-related vegetation types with treeless vegetation types. The authors refer to former 'forest', 'woodland', 'forest steppe', or 'shrubland' and show their replacement by open vegetation comprising 'alpine meadow', 'alpine steppe' or '(steppe-, desert-, pasture-) shrubland'. However, the reconstruction of vegetation formations may to a certain extent be debatable. For example, the assignation of some woody taxa to growth forms in the Rutok and Nienang diagrams is ambiguous (e.g. *Juniperus*, *Hippophae*; van Leeuwen in La Duo, 2008; Fig. 1, Table 3). Generally, they argue for shrubs, knowing that even several natural occurrences of both *Juniperus* trees (*J. tibetica*) and *Hippophae* trees (*H. rhamnoides*) presently exist in the Lhasa area. With respect to *Juniperus* they differentiate two pollen types, '*Juniperus* (small)' and '*Juniperus* (large)', which represent shrubby (*J. pingii* var. *wilsonii*) and treelike taxa (*J. convallium*, *J. tibetica*), respectively (J.F.N. van Leeuwen, Bern, pers. comm.). Using this interpretation at least the Nienang diagram shows an expansion and local occurrence of treelike *Juniperus* between 9500–6000 cal BP, which consequently reflects woodland rather than shrubland vegetation. In this regard

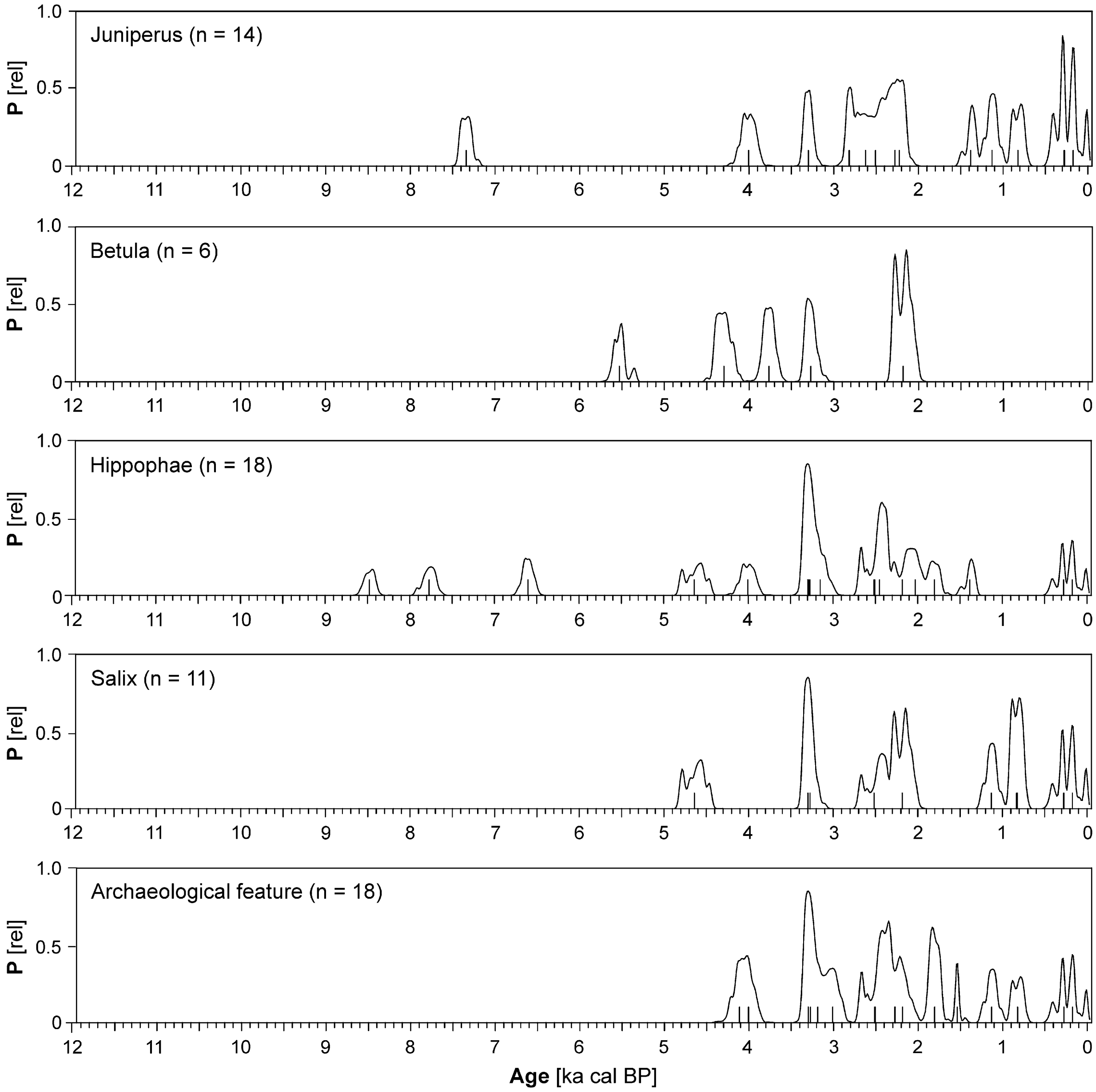


Fig. 8. Radiocarbon datings on charcoal of selected tree taxa recorded and of archaeological sites in southern Tibet thus comprising a selection of the total data set presented. The data sets used refer to both direct and assigned datings. The archaeological record is referenced in Appendix 6. It comprises 18 ^{14}C ages from 15 sites of a c. 100 to 100 km large area with Lhasa nearly in the centre.

their general reconstruction of 'shrublands' for the Early and Mid-Holocene is questionable.

Only the Damxung diagram (Fig. 1, Table 3) possibly shows the local presence of *Juniperus* trees throughout the Holocene.

For the Tibetan Plateau a Late Holocene decrease of temperature and moisture in comparison to the Early and Mid-Holocene has been concluded (e.g. He et al., 2004; Herzschuh, 2006; Morrill et al., 2006; Zhao et al., 2007; Shen et al., 2008; Yang et al., 2008; Zhu et al., 2008). There is no strong synchronism of the cooling and desiccation trend throughout the Plateau (An et al., 2006). Furthermore, most pollen records from the eastern sector reveal Early to Mid-Holocene forests and forest steppes, which were replaced by open vegetation in the Late Holocene (e.g. Yan et al., 1999; Tang et al., 2000; Frenzel, 2002; Herzschuh et al., 2005; Shen et al., 2005). The vegetation of the second part of the Holocene was considered to be of natural status and vegetation change has been assumed to be primarily caused by climatic impact. However, as the existence of 'Neolithic' economies in that area can be assumed for the last c. 4000–6000 years (Aldenderfer, 2007; Rhode et al., 2007), potential human impact (e.g. clearing of forests for expanding pastures and establishing arable land) might have strongly changed the regional vegetation cover as well (alternatively or additionally).

For several sites in the area in between the above-mentioned pollen diagrams in southern and south-eastern Tibet (Table 3), our charcoal record reveals the occurrence of trees – probably of woodlands – during the whole Holocene, forming a 'peak of burning' in the Late Holocene (see chapter '5.3. Fire and human impact'; Fig. 8). Accordingly, there is strong evidence for the assumption that this territory as a whole was formerly wooded.

5.3. Fire and human impact

In general, *macroscopic* charcoal (>200 μm) represents stand- to local-scale fires, whereas *microscopic* charcoal (<100 μm) is considered as a proxy of both local and regional burning activity (Carcaillet, 2007). However, as microscopic charcoal might originate at least partially from long-distance aeolian transport (e.g. Whitlock and Larsen, 2001; Benedict, 2002; Duffin et al., 2008), assumptions on local fires should be regarded most cautiously and need further research.

Table 3
Late Quaternary regional vegetation development derived from pollen diagrams in south-eastern and southern Tibet. Sites are mapped in Fig. 1. Nomenclature of vegetation forms (e.g. ‘forest’, ‘steppe’, ‘meadow’) follows the original references. Plant names in parentheses refer to characteristic genera and families.

Pollen diagram	Ren Co	Co Qongjiamong	Rutok	Lhasa 1	Nienang	Damxung
Reference	Shen, 2003 [a]	Shen, 2003 [a]	La Duo, 2008	Miehe et al., 2006	La Duo, 2008	Schlütz et al., 2007 [b]
Coordinates	30°44′N, 96°41′E	29°49′N, 92°32′E	29°41′N, 92°16′E	29°10′N, 91°04′E	29°43′N, 90°42′E	30°23′N, 90°53′E
Altitude (m a.s.l.)	4450	4980	4400	3650	3950	4250
Current vegetation (regional)	alpine meadow–steppe mosaic	alpine meadow	alpine meadow–shrubland	degraded pasture–shrubland	alpine meadow–desert shrubland	alpine sedge mat with some *Juniperus* trees
Site situation	Lake basin	lake basin	peatland in river valley	peatland in river valley	foothill depression	peatland in river valley
Vegetation development (regional)	0–4500 cal BP, hiatus	0–5000 cal BP, alpine meadow (Cyperaceae)	0–300 cal BP, steppe-shrubland (Gramineae, *Juniperus*, *Hippophae*)	0–600 cal BP, degraded pasture-shrubland (*Artemisia, Juniperus*)	0–600 cal BP, open steppe-desert shrubland (*Artemisia*)	0–1200 cal BP, degraded alpine mat-woodland (Cyperaceae, *Juniperus*)
	4500–6300 cal BP, alpine steppe (*Artemisia*)	5000–8000 cal BP, forest (*Pinus, Betula*)	300–6800 cal BP, hiatus	600–4700 cal BP, shrubland (*Hippophae*, *Betula*, *Artemisia*)	600–3500 cal BP, hiatus	1200–8500 cal BP, alpine mat (Cyperaceae)
	6300–11,000 cal BP, forest steppe (*Betula*, *Abies*, *Picea*)	8000–13,500 cal BP, alpine meadow (Cyperaceae)	6800–11,300 cal BP, meadow-shrubland (Gramineae, Cyperaceae, *Hippophae*, *Juniperus*, *Betula*, *Salix*)	4700–5000 cal BP, woodland (*Juniperus*, *Betula*, *Hippophae*, *Rhododendron*)	3500–6000 cal BP, dry steppe-shrubland (*Artemisia*, Gramineae, Chenopodiaceae, *Juniperus*, *Salix*, *Betula*)	
	11,000–14,000 cal BP, alpine steppe (Chenopodiaceae)	13,500–14,000 cal BP, alpine steppe (*Artemisia*)	11,300–11,900 cal BP, desert steppe-shrubland (*Artemisia*)		6000–9500 cal BP, temp. steppe-shrubland (*Artemisia*, Gramineae, *Hippophae*, *Juniperus*, *Salix*, *Betula*)	8500–13,000 cal BP, steppe-shrubland (Poaceae, *Hippophae*, *Myricaria*, *Juniperus*)
			11,900–13,000 cal BP, temp. steppe-shrubland (Gramineae, Cyperaceae, *Hippophae*, *Salix*, *Juniperus*)		9500–10,000 cal BP, steppe-shrubland (*Artemisia*, Gramineae, *Hippophae*, *Salix*)	

[a] A simplified version of this diagram is available in Tang et al. (2000).
[b] The original diagram was published in German. A simplified English version of this diagram is available in Kaiser et al. (2008).

The distribution of ^{14}C ages in our macroscopic charcoal samples shows no continuous record of burning events in southern and south-eastern Tibet throughout the Holocene. Instead, burning conspicuously cumulated in the Late Holocene about 5700–0 cal BP (Figs. 7 and 8).

The few regional records of Late Quaternary microscopic charcoal deposition yielded by pollen-analytical studies provide incomplete insights so far due to the fragmentary preservation of Late Holocene sediments (hiatuses). The records from Rutok and Nienang comprise the intervals c. 13,000–6800 and c. 10,000–3500 cal BP, showing charcoal peaks at 11,300–7500 and c. 10,000–6000 cal BP, respectively (van Leeuwen in La Duo, 2008). The completely preserved Damxung sequence (base c. 13,000 cal BP) shows peaks at c. 10,500–9000 and 3000–2000 cal BP (Schlütz et al., 2007; Kaiser et al., 2008). Consequently, these studies reveal fire activity in the region throughout the Holocene. However, the proper identification of distinct ‘fire periods’ (peaking) and their reasons for the whole Holocene remains unclear.

The question arises, what caused the Late Holocene peak of our macroscopic charcoal dataset – an increase in human-induced burning activity or climate-induced natural changes (increase of natural ignitions, increasing potential of fire spread)? An answer initially requires a consideration of the *present* fire regime in the region.

So far, knowledge on the fire dynamics on the Tibetan Plateau is poor. An overview of recent forest fuel and wildfire characteristics of southeast Tibet has shown that – although, in general, the ‘fire cycle’ is long (c. 17,200 years) and the ‘fire probability’ is very low ($P = 0.00006$) – specific regional ecosystems (esp. coniferous forests) tend to have a high fire incidence especially in the dry winter season, which is obviously caused by current *human* impact (Wang et al., 2007). A case study from the Jiuzhaigou Nature Reserve, north-eastern Plateau, revealed a graded impact of fire on present-day forest types on *south-facing* slopes (Winkler, 2000). According to field observations, interviews and historical data, the local population utilises fire as a means of extending and maintaining livestock pasture, favouring the lower and upper altitudes. Obviously, after clearing of the primary forests, continuous grazing and repeated burning prevents forest regrowth there at present.

Generally speaking, all episodically or periodically *dry* ecosystems throughout the world – forests, woodlands, (dwarf-) shrublands, grasslands – are considered to be fire-sensitive (e.g. Berrio et al., 2002; Hobbs, 2002; Brown et al., 2005; Carcaillet et al., 2007). In the semiarid (monsoonal) climate of southern and south-eastern Tibet any shift to higher precipitations and temperatures during the wet season (summer) will provide more phytomass (fuel) to be set on fire during the dry season (winter) influencing the possibility of fire spread. The summer is the only season with potential natural causes of fire (lightning). However, based on our own observational data of the last c. 20 years from the region, we have no evidence of naturally caused fires during the frequently occurring thunderstorms: Lightning has always been followed by torrential rains. Furthermore, in an ecosystem (1) not characterised by the prevalence of pyrophytes and (2) with a proven present-day climate suitable for tree growth (see above), the human factor is the more likely explanation for the accumulation of Late Holocene burning events. A similar conclusion was drawn by Meyer et al. (in press) for the adjacent northwest Bhutan. In general, the significant increase in fire frequency accompanying human activities is a known phenomenon and is often detected in palaeoenvironmental studies (e.g. Carcaillet and Brun, 2000; Burney and Burney, 2003; Huang et al., 2006).

Our charcoal data (record of trees and shrubs) and the present-day vegetation of the sites (mostly tree- and shrubless) indicates a probable link between fire activity and vegetation change. Several factors may be involved in fire dynamics and mediate vegetation changes (e.g. climatic conditions, such as frequency of lightning and length of drought; structure of tree- and ground-cover; availability of combustible biomass; recovery potential of woody species; occurrence of herbivores; several human activities, such as burning, grazing, tree cutting; e.g. Pyne, 1995; Johnson, 1996; Higgins et al., 2000; Gillson, 2004; Long et al., 2007). However, reliable data is largely lacking on the regional characteristics of these factors in the past. There are no palaeoecological data on fire-driven plant community changes in our study area so far. An example from a nearby comparable environment is from Lake Rukche in the central Nepalese Himalayas (c. 600 km to the southwest, 3500 m a.s.l.) showing fire-affected replacement of dense oak forest by open pine forest at c. 2850 cal BP, interpreted as initiated by man (Schlütz and Zech, 2004). Furthermore, the joint occurrence of macroscopic charcoal and cereal pollen grains at c. 4500 cal BP in northwest Bhutanese palaeosols (c. 200 km to the south, 3700–4000 m a.s.l.) was interpreted as evidence for human use of fire and for forest clearing due to Neolithic agriculture (Meyer et al., in press).

There is some archaeological evidence for early human impact both in south-eastern and southern Tibet. The most important 'Neolithic' sites – implying (at least locally) a strong human influence on the landscape by sedentary settlement and agriculture – are Kha rub/Karou next to Chamdo (5732 ± 114 to 4372 ± 117 cal BP) and Chugong/Qugong next to Lhasa (c. 3700 cal BP; Aldenderfer, 2007; Fig. 1). Further 'Neolithic' sites between the Mekong River and the Nyainqentanglha Range have been only initially investigated so far, yielding radiocarbon ages from 4162 ± 130 to 3010 ± 82 cal BP (Fu et al., 2000; Kaiser et al., 2006, 2009, in press-a; Aldenderfer, 2007).

In the Lhasa area, our charcoal record is variously linked with local archaeological evidence comprising a total of 10 spectra (Figs. 1 and 8, Appendices 3 and 6). In general, archaeological charcoal may be considered as a proxy of past vegetation, provided that the database is large enough (Carcaillet, 2007) and/or that diversity of species in a study area is poor. Human collection of firewood may affect the reflection of the past composition and abundance of woody species (Shackleton and Prins, 1992). However, both the charcoal spectra and the present-day vegetation of the Lhasa area reflect a species-poor woody vegetation with the same taxa occurring (e.g. *Juniperus*, *Hippophae*, *Betula* and *Salix*). Accordingly, the charcoal taxa can be considered as elements of the local flora. The charcoal-bearing palaeosols of six profiles contained numerous archaeological remains as well (pot shards, bones, stone flakes, in some cases slag), thus representing occupation layers. Most of the datings are scattered over the last c. 4000–2000 years (4005 ± 79–1803 ± 61 cal BP; GAR 1, QUG 1, FAN 1, GUZ 1b, BRI 1); only one age attains the recent past (175 ± 111 cal BP; DRE 2). Two burial mounds (GHU 1, LSW 3) could be dated using charcoal and fossil wood, giving ages of 2276 ± 72 and 1806 ± 53 cal BP. Possibly, even charred Poaceae seeds (a cereal, probably barley) from two charcoal spectra (SAI 2, GHU 2) can be associated with human cultivation (see a further regional record made by Fu et al., 2000). The seeds occur together with charcoal of *Juniperus* and *Betula*, respectively. Finally, in the Reting area, buried soils from anthropogenic (field-) terraces could be dated to 1131 ± 59 and 826 ± 64 cal BP (RET 1, RET 8) by means of charcoal, giving first chronological data for this regionally widespread and now mostly abandoned land-use feature. Strikingly, if we include a dated human skeleton from Lhasa-Drepung (2272 ± 68 cal BP; Kaiser et al., 2009, in press-a) and further archaeological, dendroecological and geomorphic evidence from the Lhasa area (Kaiser, 2004; Kaiser et al., 2006; Aldenderfer, 2007; Bräuning, 2007) most datings are scattered over the time interval c. 3500–1500 cal BP, presumably revealing an intensified occupation hence human impact on the Lhasa area in that time (Fig. 8).

According to the investigation of colluvial sediments in the Kyichu Valley and its tributaries, it can be hypothesised that the barren valley slopes in that area were primarily formed by a Late Pleistocene erosion phase (probably triggered by ice-age climatic effects) followed by a secondary, probably human-induced Late Holocene erosion phase (Kaiser et al., 2006; Kaiser et al., in press-a). A similar conclusion can be drawn for this area from the investigation of aeolian sands showing local, probably human-induced Late Holocene aeolian activity (Kaiser et al., 2009).

To sum up, the synchronous record of Late Holocene human occupation (archaeological evidence), burning events (charcoal dating), vegetation changes (charcoal determination, pollen record) and erosional processes (dated colluvial and aeolian deposits) suggest that above all man might have induced the environmental changes from woodlands to desertic rangelands in southern Tibet.

5.4. *Regional pedoanthracological potential*

Only in recent years have the first systematically determined charcoal *spectra* from southern and north-eastern Tibet been published in international journals (Kaiser et al., 2006, 2007, 2009, in press-a), succeeding initial studies on the south-eastern Plateau (Iwata et al., 1993; Iwata, 1994). Further but incompletely reported charcoal determinations are available from archaeological sites at Qinghai Lake in northeast Tibet (Madsen et al., 2006; Rhode et al., 2007). Fossil wood – usually botanically undetermined – has been extracted on the Plateau mostly from moraines, lacustrine sediments and archaeological sites so far (e.g. Xu et al., 2003; Kuhle, 2005; Yang et al., 2008). In general, a search for studies using soil-derived charcoal in central Asia, the Himalayas and further Chinese mountain areas (e.g. Saijo, 1993; Rost, 2001; Saijo and Tanaka, 2002; Byers, 2005; Wang et al., 2005; Huang et al., 2006; Miehe et al., 2007a; Srivastava et al., 2007; Jiang et al., 2008; Power et al., 2008; Meyer et al., in press) reveals that pedoanthracology is not a still well-established palaeoecological method in these regions.

Our study presents the largest regional data set of both botanically determined and geochronologically dated charcoal available from the Tibetan Plateau so far. Most of the palaeosols recorded contain charcoal. In particular, the buried Histosol (peat layer) of profile CHU 5 – a fossil floodplain site with a large quantity of both charcoal and well-preserved fossil wood – offers a promising archive for detailed palaeoenvironmental studies. Similar wet-site palaeo-positions in a present-day 'dry environment' have been repeatedly recorded (CHS 1, DAV 2, CNG 1, CNG 2). Normally, the palaeosols sampled had strongly dispersed and fragmented charcoal only. As rather thick layers were sampled for this study (mean = 24 cm), a future check for a possible higher botanical and temporal resolution is needed.

In summary, the pedoanthracological potential of southern and south-eastern Tibet seems promising for future high-resolution studies. Especially data for specifying the prehistoric and historic fire regimes would be a tremendous asset, both for regional palaeoecology and human settlement history.

6. Conclusions

(1) In southern and south-eastern Tibet, charcoal frequently occurs in palaeosols, various sediments and artificial structures forming an important palaeoecological proxy. In particular dry-site palaeosols regularly contain strongly dispersed and fragmented macroscopic charcoal. Moreover, wet-site palaeosols bear well-preserved fossil wood.

(2) Most taxa determined represent (dwarf-) shrubs, followed by trees and herbs/grasses. The genera *Hippophae* and *Juniperus* statistically prevail, representing tree species in our study area.

(3) Charcoal of *Juniperus* mainly occurs on or below south-facing slopes, whereas *Betula* is mainly associated with a general north exposure. In contrast, the (partly) phreatophytic taxa *Hippophae* and *Salix* show no clear prevalence of an orientation.
(4) There is a strong disparity of prevalently Holocene ages in comparison to a few Pleistocene ages. Most datings are scattered over the Late Holocene (c. 5700–0 cal BP).
(5) The charcoal and fossil wood spectra analysed give evidence for a Late Holocene change in southern Tibet from a tree- and shrub-dominated vegetation to the present plant cover widely consisting of sparse grasses, herbs and dwarf-shrubs. It is assumed that the Late Holocene disappearance of woodlands has been primary caused by humans.

Acknowledgements

We are grateful to Gen Dun, Tibet-Museum Lhasa, and Shargan Wangdwei, Tibetan Authority of Cultural Relics Lhasa, whose knowledge in regional archaeology enabled us to sample some profiles at archaeological sites. The fieldwork was partly carried out under the auspices of the Lhasa-Marburg University partnership; special thanks go to our colleagues La Duo and Tsering Dorgeh for their untiring support. We also thank Christiane Enderle, Marburg University, for preparation of some figures, James Fanning, Greifswald University, for improving the English, as well as John B. Birks, Torstein Solhøy, both Bergen University, and Jacqueline F.N. van Leeuwen, Bern University, for stimulating discussions. Furthermore, we owe M. Aldenderfer, University of Arizona, O. Nelle, Kiel University, and two anonymous reviewers helpful comments on an earlier version of this paper. Research and the printing of the colour figures were kindly supported by the German Research Foundation (DFG grants KA 2713/1-1, Mi 271/15, Mi 271/18-1).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.quascirev.2009.02.016.

References

Aldenderfer, M., 2007. Modeling the Neolithic on the Tibetan Plateau. Developments in Quaternary Sciences 9, 151–165.
Alon, D., Mintz, G., Cohen, I., Weiner, S., Boaretto, E., 2002. The use of Raman spectroscopy to monitor the removal of humic substances from charcoal: quality control for ^{14}C dating of charcoal. Radiocarbon 44, 1–11.
An, C.B., Feng, Z.D., Barton, L., 2006. Dry or humid? Mid-Holocene humidity changes in arid and semi-arid China. Quaternary Science Reviews 25, 351–361.
Atlas of Tibet Plateau, 1990. Edited by the Institute of Geography, Chinese Academy of Sciences. Beijing (in Chinese).
Benedict, J., 2002. Eolian deposition of forest-fire charcoal above tree limit, Colorado Front Range, U.S.A.: potential contamination of AMS radiocarbon samples. Arctic, Antarctic, and Alpine Research 34, 33–37.
Berglund, B.E., Ralska-Jasiewiczowa, M., 1986. Pollen analysis and pollen diagrams. In: Berglund, B.E. (Ed.), Handbook of Holocene Palaeoecology and Palaeohydrology. Wiley, Chichester, pp. 455–484.
Berrio, J.C., Hooghiemstra, H., Marchant, R., Rangel, O., 2002. Late-glacial and Holocene history of the dry forest area in the south Colombian Cauca Valley. Journal of Quaternary Science 17, 667–682.
Beug, H.-J., Miehe, G., 1999. Vegetation history and human impact in the Eastern Central Himalaya (Lang-tang and Helambu, Nepal). In: Dissertationes Botanicae, vol. 318. Borntraeger, Stuttgart.
Bräuning, A., 2007. Dendroecological studies on the Tibetan Plateau. Implications of relict juniper forests for climate change and cultural history. Geographische Rundschau International Edition 3, 38–43.
Brown, K.J., Clark, J.S., Grimm, E.C., Donovan, J.J., Mueller, P.G., Hansen, B.C.S., Stefanova, I., 2005. Fire cycles in North American interior grasslands and their relation to prairie drought. Proceedings of the National Academy of Sciences USA 102, 8865–8870.
Burney, L.P., Burney, D.A., 2003. Charcoal stratigraphies for Kaua'i and the timing of human arrival. Pacific Science 57, 211–226.
Byers, A., 2005. Contemporary human impacts on alpine ecosystems in the Sagarmatha (Mt. Everest) National Park, Khumbu, Nepal. Annals of the Association of American Geographers 95, 112–140.
Carcaillet, C., 2007. Charred particle analysis. In: Elias, S.A. (Ed.), Encyclopedia of Quaternary Science, vol. 2. Elsevier, Amsterdam, pp. 1582–1593.
Carcaillet, C., Thinon, M., 1996. Pedoanthracological contribution to the study of the evolution of the upper treeline in the Maurienne valley (North French Alps): methodology and preliminary data. Review of Palaeobotany and Palynology 91, 399–416.
Carcaillet, C., Brun, J., 2000. Changes in landscape structure in the northwestern Alps over the last 7000 years: lessons from soil charcoal. Journal of Vegetation Science 11, 705–714.
Carcaillet, C., Bergman, I., Delorme, S., Hörnberg, G., Zackrisson, O., 2007. Long-term fire frequency not linked to prehistoric occupations in northern Swedish boreal forest. Ecology 88, 465–477.
Carnelli, A.L., Theurillat, J.-P., Thinon, M., Vadi, G., Talon, B., 2004. Past uppermost tree limit in the Central European Alps (Switzerland) based on soil and soil charcoal. The Holocene 14, 393–405.
Di Pasquale, G., Marziano, M., Impagliazzo, S., Lubritto, C., Natale, A.D., Bader, M.Y., 2008. The Holocene treeline in the northern Andes (Ecuador): first evidence from soil charcoal. Palaeogeography, Palaeoclimatology, Palaeoecology 259, 17–34.
Domrös, M., Peng, G., 1988. The Climate of China. Springer, Berlin.
Duffin, K.I., Gillson, L., Willis, K.J., 2008. Testing the sensitivity of charcoal as an indicator of fire events in savanna environments: quantitative predictions of fire proximity, area and intensity. The Holocene 18, 279–291.
Farjon, A., 2005. A Monograph of Cupressaceae and Sciadopitys. Royal Botanic Gardens, Kew.
Feng, Z.D., Tang, L.Y., Wang, H.B., Ma, Y.Z., Liu, K.B., 2006. Holocene vegetation variations and the associated environmental changes in the western part of the Chinese Loess Plateau. Palaeogeography, Palaeoclimatology, Palaeoecology 241, 440–456.
Frenzel, B., 2002. History of flora and vegetation during the Quaternary. Progress in Botany 63, 368–385.
Fu, D.X., Xu, T.W., Feng, Z.Y., 2000. The ancient carbonized barley (*Hordeum vulgare* L. Var. *nudum*) kernel discovered in the middle of Yalu Tsanypo river basin in Tibet. Southwest China Journal of Agricultural Sciences 13, 38–41.
Geyh, M., 2005. ^{14}C dating – still a challenge for users? Zeitschrift für Geomorphologie Supplement 139, 63–86.
Gillson, L., 2004. Testing non-equilibrium theories in savannas: 1400 years of vegetation change in Tsavo National Park, Kenya. Ecological Complexity 1, 281–298.
Havinga, A.J., 1984. A 20-year experimental investigation into the differential corrosion susceptibility of pollen and spores in various soil types. Pollen et Spores 26, 541–558.
He, Y., Theakstone, W.H., Zhang, Z.L., Zhang, D.A., Yao, T.D., Chen, T.O., Shen, Y.P., Pang, H.X., 2004. Asynchronous Holocene climatic change across China. Quaternary Research 61, 52–63.
Herzschuh, U., 2006. Palaeo-moisture evolution in monsoonal Central Asia during the last 50,000 years. Quaternary Science Reviews 25, 163–178.
Herzschuh, U., Zhang, C., Mischke, S., Herzschuh, R., Mohammadi, F., Mingram, B., Kürschner, H., Riedel, F., 2005. A late Quaternary lake record from the Qilian Mountains (NW China), evolution of the primary production and the water depth reconstructed from macrofossil, pollen, biomarker and isotope data. Global and Planetary Change 46, 361–379.
Higgins, S.I., Bond, W.J., Trollope, W.S., 2000. Fire, resprouting and variability: a recipe for grass–tree coexistence in savannas. Journal of Ecology 88, 213–229.
Hoadley, R.B., 1990. Identifying Wood. Accurate Results with Simple Tools. The Taunton Press, Newtown.
Hobbs, R.J., 2002. Fire regimes in temperate woodlands. In: Bradstock, R., Williams, J., Gill, M. (Eds.), Flammable Australia: The Fire Regimes and Biodiversity of a Continent. Cambridge University Press, Cambridge, pp. 305–326.
Holzner, W., Kriechbaum, M., 1998. Man's impact on the vegetation and landscape in the inner Himalaya and Tibet. In: Elvin, M., Liu, T.J. (Eds.), Sediments of Time, Environment and Society in Chinese History. Cambridge University Press, Cambridge, pp. 53–106.
Huang, C.C., Pang, J.L., Chen, S., Su, H.X., Han, J.A., Cao, Y.F., Zhao, W.Y., Tan, Z.H., 2006. Charcoal records of fire history in the Holocene loess-soil sequences over the southern Loess Plateau of China. Palaeogeography, Palaeoclimatology, Palaeoecology 239, 28–44.
Iwata, S., Ageta, Y., Ohata, T., 1993. Radiocarbon ages of woods and buried soil layers and their significance in the Potechu Valley, southeastern Tibet. Summaries of Researches using AMS at Nagoya University 4, 27–35 (in Japanese).
Iwata, S., 1994. Buried soil layers and deforestation in the southern Part of the Himalaya–Tibet massif. Bulletin of the Faculty of Humanities and Social Sciences Mie University 11, 45–62 (in Japanese).
Jiang, W.Y., Leroy, S.A.G., Ogle, N., Chu, G.Q., Wang, L., Liu, J.Q., 2008. Natural and anthropogenic forest fires recorded in the Holocene pollen record from a Jinchuan peat bog, northeastern China. Palaeogeography, Palaeoclimatology, Palaeoecology 261, 47–57.
Johnson, E.A., 1996. Fire and Vegetation Dynamics: Studies from the North American Boreal Forest. Cambridge University Press, Cambridge, 143 pp.
Kaiser, K., 2004. Pedogeomorphological transect studies in Tibet: implications for landscape history and present-day dynamics. Prace Geograficzne 200, 147–165.

Kaiser, K., Miehe, G., Schoch, W.H., Zander, A., Schlütz, F., 2006. Relief, soil and lost forests: Late Holocene environmental changes in southern Tibet under human impact. Zeitschrift für Geomorphologie Supplement 142, 149–173.

Kaiser, K., Schoch, W.H., Miehe, G., 2007. Holocene paleosols and colluvial sediments in Northeast Tibet (Qinghai Province, China): properties, dating and paleoenvironmental implications. Catena 69, 91–102.

Kaiser, K., Miehe, G., Barthelmes, A., Ehrmann, O., Scharf, A., Schult, M., Schlütz, F., Adamczyk, S., Frenzel, B., 2008. Turf-bearing topsoils on the central Tibetan Plateau, China: pedology, botany, geochronology. Catena 73, 300–311.

Kaiser, K., Lai, Z.P., Schneider, B., Reudenbach, C., Miehe, G., Brückner, H., 2009. Stratigraphy and palaeoenvironmental implications of Pleistocene and Holocene aeolian sediments in the Lhasa area, southern Tibet (China). Palaeogeography, Palaeoclimatology, Palaeoecology 271, 329–342.

Kaiser, K., Lai, Z.P., Schneider, B., Schoch, W.H., Shen, X.H., Miehe, G., Brückner, H. Sediment sequences and paleosols in the Kyichu Valley, southern Tibet (China), indicating Late Quaternary environmental changes. Island Arc, in press-a.

Kaiser, K., Lai, Z.P., Schneider, B., Junge, F.W. Late Pleistocene genesis of the middle Yarlung Zhangbo Valley, southern Tibet (China), as deduced by sedimentological and luminescence data. Quaternary Geochronology, in press-b.

Kuhle, M., 2005. Glacial geomorphology and ice ages in Tibet and the surrounding mountains. Island Arc 14, 346–367.

La Duo, 2008. Studies in the Ecology and Palaeoecology of the Lhasa Valley, Tibet Autonomous Region, China. PhD thesis, Department of Biology, University of Bergen (Norway).

Lehmkuhl, F., Klinge, M., Lang, A., 2002. Late Quaternary glacier advances, lake level fluctuations and aeolian sedimentation in Southern Tibet. Zeitschrift für Geomorphologie Supplement 126, 183–218.

Long, C.J., Whitlock, C., Bartlein, P.J., 2007. Holocene vegetation and fire history of the Coast Range, western Oregon, USA. The Holocene 17, 917–926.

Luo, T.X., Pan, Y.D., Ouyang, H., Shi, P.L., Luo, J., Yu, Z.L., Lu, Q., 2004. Leaf area index and net primary productivity along subtropical to alpine gradients in the Tibetan Plateau. Global Ecology and Biogeography 13, 345–358.

Luo, T.X., Brown, S., Pan, Y.D., Shi, P.L., Ouyang, H., Yu, Z.L., Zhu, H.Z., 2005. Root biomass along sub-tropical to alpine gradients: global implication from Tibetan transect studies. Forest Ecology and Management 206, 349–363.

Lynch, J.A., Clark, J.S., Stocks, B.J., 2004. Charcoal production, dispersal and deposition from the Fort Providence experimental fire: interpreting fire regimes from charcoal records in boreal forests. Canadian Journal of Forest Research 34, 1642–1656.

Madsen, D.B., Ma, H.Z., Brantingham, P.J., Gao, X., Rhode, D., Zhang, H.Y., Olsen, J.W., 2006. The Late Upper Paleolithic occupation of the northern Tibetan Plateau margin. Journal of Archaeological Science 33, 1433–1444.

Meng, L.H., Yang, R., Abbott, R.J., Miehe, G., Hu, T.H., Liu, J.Q., 2008. Mitochondrial and chloroplast phylogeography of *Picea crassifolia* Kom. (Pinaceae) in the Qinghai–Tibetan Plateau and adjacent highlands. Molecular Ecology 16, 4128–4137.

Meyer, M.C., Hofmann, C.-C., Gemmell, A.M.D., Haslinger, E., Häusler, H., Wangda, D. Holocene glacier fluctuations and migration of Neolithic yak pastoralists into the high valleys of northwest Bhutan. Quaternary Science Reviews, in press.

Miehe, G., Winiger, M., Böhner, J., Zhang, Y.L., 2001. The climatic diagram map of High Asia. Purpose and concepts. Erdkunde 55, 94–97.

Miehe, G., Miehe, S., Koch, K., Will, M., 2003. Sacred forests in Tibet. Using geographical information systems for forest rehabilitation. Mountain Research and Development 23, 324–328.

Miehe, G., Miehe, S., Schlütz, F., Kaiser, K., Duo, La, 2006. Palaeoecological and experimental evidence of former forests and woodlands in the treeless desert pastures of Southern Tibet (Lhasa, A.R. Xizang, China). Palaeogeography, Palaeoclimatology, Palaeoecology 242, 54–67.

Miehe, G., Schlütz, F., Miehe, S., Opgenoorth, L., Cermak, J., Samiya, R., Jäger, E.J., Wesche, K., 2007a. Mountain forest islands and Holocene environmental changes in Central Asia: A case study from the southern Gobi Altay, Mongolia. Palaeogeography, Palaeoclimatology, Palaeoecology 250, 150–166.

Miehe, G., Miehe, S., Vogel, J., Co, Sonam, Duo, La, 2007b. Highest treeline in the northern hemisphere found in southern Tibet. Mountain Research and Development 27, 169–173.

Miehe, G., Miehe, S., Will, M., Opgenoorth, L., Duo, La, Dorgeh, Tsering, Liu, J.Q., 2008a. An inventory of forest relics in the pastures of Southern Tibet (Xizang A.R., China). Plant Ecology 194, 157–177.

Miehe, G., Miehe, S., Kaiser, K., Liu, J.Q., Zhao, X.Q., 2008b. Status and dynamics of the *Kobresia pygmaea* ecosystem on the Tibetan Plateau. Ambio 37, 272–279.

Mosbrugger, V., Li, X.M., Yao, T.D., 2007. The Tibetan Plateau during the Holocene – forests or no forests? Quaternary International 167–168, 290.

Morrill, C., Overpeck, J.T., Cole, J.E., Liu, K.B., Shen, C.M., Tang, L.Y., 2006. Holocene variations in the Asian monsoon inferred from the geochemistry of lake sediments in central Tibet. Quaternary Research 65, 232–243.

Ni, J.A., 2000. A simulation of biomes on the Tibetan Plateau and their responses to global climate change. Mountain Research and Development 20, 80–89.

Power, M.J., Marlon, J., Ortiz, N., Bartlein, P.J., Harrison, S.P., Mayle, F.E., Ballouche, A., Bradshaw, R.H.W., Carcaillet, C., Cordova, C., Mooney, S., Moreno, P.I., Prentice, I.C., Thonicke, K., Tinner, W., Whitlock, C., Zhang, Y., Zhao, Y., Ali, A.A., Anderson, R.S., Beer, R., Behling, H., Briles, C., Brown, K.J., Brunelle, A., Bush, M., Camill, P., Chu, G.Q., Clark, J., Colombaroli, D., Connor, S., Daniau, A.L., Daniels, M., Dodson, J., Doughty, E., Edwards, M.E., Finsinger, W., Foster, D., Frechette, J., Gaillard, M.J., Gavin, D.G., Gobet, E., Haberle, S., Hallett, D.J., Higuera, P., Hope, G., Horn, S., Inoue, J., Kaltenrieder, P., Kennedy, L., Kong, Z.C., Larsen, C., Long, C.J., Lynch, J., Lynch, E.A., McGlone, M., Meeks, S., Mensing, S., Meyer, G., Minckley, T., Mohr, J., Nelson, D.M., New, J., Newnham, R., Noti, R., Oswald, W., Pierce, P.J., Richard, H., Rowe, C., Goni, M.F.S., Shuman, B.N., Takahara, H., Toney, J., Turney, C., Urrego-Sanchez, D.H., Umbanhowar, C., Vandergoes, M., Vanniere, B., Vescovi, E., Walsh, M., Wang, X., Williams, N., Wilmshurst, J., Zhang, J.H., 2008. Changes in fire regimes since the Last Glacial Maximum: an assessment based on a global synthesis and analysis of charcoal data. Climate Dynamics 30, 887–907.

Pyne, S.J., 1995. World Fire. The Culture of Fire on Earth. University of Washington Press, Seattle, 384 pp.

Ren, G.Y., 2000. Decline of the mid- to late Holocene forests in China: climatic change or human impact? Quaternary Science Reviews 15, 273–281.

Ren, G.Y., 2007. Changes in forest cover in China during the Holocene. Vegetation History and Archaeobotany 16, 119–126.

Ren, G.Y., Beug, H.-J., 2002. Mapping Holocene pollen data and vegetation of China. Quaternary Science Reviews 21, 1395–1422.

Rhode, D., Zhang, H.Y., Madsen, D.B., Gao, X., Brantingham, P.J., Ma, H.Z., Olsen, J.W., 2007. Epipaleolithic/early Neolithic settlements at Qinghai Lake, western China. Journal of Archaeological Science 34, 600–612.

Rost, K.T., 2001. Late Holocene loess deposits and dust accumulation in the alpine meadow belt of the Wutai Shan, China. Quaternary International 76–77, 85–92.

Saijo, K., 1993. A ^{14}C date of buried charcoal fragments found in the middle mountains of Nepal. Mountain Research and Development 13, 377–378.

Saijo, K., Tanaka, S., 2002. Paleosols of middle Holocene age in the Thakkhola basin, central Nepal, and their paleoclimatic significance. Journal of Asian Earth Sciences 21, 323–329.

Scharf, A., Uhl, T., Luppold, W., Rottenbach, A., Kritzler, K., Ohneiser, A., Kretschmer, W., 2007. Status report on the Erlangen AMS facility. Nuclear Instruments and Methods in Physics Research B 259, 50–56.

Schlütz, F., Zech, W., 2004. Palynological investigations on vegetation and climate change in the Late Quaternary of Lake Rukche area, Gorkha Himal, Central Nepal. Vegetation History and Archaeobotany 13, 81–90.

Schlütz, F., Lehmkuhl, F., 2009. Holocene climatic change and the nomadic Anthropocene in Eastern Tibet: palynological and geomorphological results from the Nianbaoyeze Mountains. Quaternary Science Reviews 28, 1449–1471.

Schlütz, F., Miehe, G., Lehmkuhl, F., 2007. Zur Geschichte des größten alpinen Ökosystems der Erde – Palynologische Untersuchungen zu den *Kobresia*-Matten SE-Tibets. Berichte der Reinhold-Tüxen-Gesellschaft 19, 23–36.

Schoch, W.H., 1986. Wood and charcoal analysis. In: Berglund, B.E. (Ed.), Handbook of Holocene Palaeoecology and Palaeohydrology. Wiley, Chichester, pp. 619–626.

Schoch, W., Heller, I., Kienast, F., 2004. Wood anatomy of central European species. Online database: http://www.woodanatomy.ch.

Schweingruber, F.H., 1990. Anatomie europäischer Hölzer. Haupt, Bern.

Seppä, H., 2007. Pollen analysis, principles. In: Elias, S.A. (Ed.), Encyclopedia of Quaternary Science, vol. 3. Elsevier, Amsterdam, pp. 2486–2497.

Shen, C.M., 2003. Millennial-scale Variations and Centennial-scale Events in the Southwest Asian Monsoon: Pollen Evidence from Tibet. PhD thesis, Louisiana State University, Baton Rouge (USA).

Shen, C.M., Liu, K.B., Morrill, C., Overpeck, J.T., Peng, J.L., Tang, L.Y., 2008. Ecotone shift and major droughts during the Mid-Late Holocene in the central Tibetan Plateau. Ecology 89, 1079–1088.

Shen, J., Liu, X.Q., Wang, S.M., Matsumoto, R., 2005. Palaeoclimatic changes in the Qinghai Lake area during the last 18,000 years. Quaternary International 136, 131–140.

Shackleton, C.M., Prins, F., 1992. Charcoal analysis and the “principle of least effort” – a conceptual model. Journal of Archaeological Science 19, 631–637.

Song, M.H., Zhou, C.P., Hua, O.Y., 2004. Distributions of dominant tree species on the Tibetan Plateau under current and future climate scenarios. Mountain Research and Development 24, 166–173.

Srivastava, P., Kumar Singh, A., Parkash, B., Singh, A.K., Rajak, M.K., 2007. Paleoclimatic implications of micromorphic features of Quaternary paleosols of NW Himalayas and polygenetic soils of the Gangetic Plains – a comparative study. Catena 70, 169–184.

Sugita, S., 2007. Theory of quantitative reconstruction of vegetation I: pollen from large sites reveals regional vegetation composition. Holocene 17, 229–241.

Tang, L.Y., Shen, C.M., Liu, K.B., Overpeck, J.T., 2000. Changes in South Asian monsoon: new high-resolution paleoclimatic records from Tibet, China. Chinese Science Bulletin 45, 87–90.

Tessier, L., de Beaulieu, J.-L., Coúteaux, M., Édouard, J.-L., Ponel, P., Rolando, C., Thinon, M., Thomas, A., Tobolski, K., 1993. Holocene palaeoenvironment at the timberline in the Alps (Taillefer Massif, French Alps): a multidisciplinary approach. Boreas 22, 244–254.

Thomas, A., Chen, S., 2002. Landwirtschaft und klimatische Trends im zentralen Yarlung Tsangpo-Tal, Tibet. Erdkunde 56, 371–384.

Tinner, W., Ammann, B., Germann, P., 1996. Treeline fluctuations recorded for 12500 years by soil profiles, pollen and plant macrofossils in the Central Swiss Alps. Arctic and Alpine Research 28, 131–147.

Wang, M.Y., Shu, L.F., Zhao, F.J., Tian, X.R., 2007. Forest fuel characteristics and the impacts of climate change on forest fires in southeast Tibet. In: Proceedings of the 4th International Wildland Fire Conference, Sevilla, Spain, 13–18 May 2007, pp. 1–9.

Wang, X., Peng, P.A., Ding, Z.L., 2005. Black carbon records in Chinese Loess Plateau over the last two glacial cycles and implications for paleofires. Palaeogeography, Palaeoclimatology, Palaeoecology 223, 9–19.

Weninger, B., Jöris, O., Danzeglocke, U., 2007. CalPal-2007. Cologne radiocarbon calibration & palaeoclimate research package. Available from: http://www.calpal.de.

Whitlock, C., Larsen, C., 2001. Charcoal as a fire proxy. In: Smol, J.P., Birks, H.J.B., Last, W.M. (Eds.), Tracking Environmental Change Using Lake Sediments. Kluwer, Dordrecht, pp. 75–97.

Winkler, D., 2000. Patterns of forest distribution and the impact of fire and pastoralism in the forest region of Tibet. Marburger Geographische Schriften 135, 201–227.

Wu, C., 1983–1987. Flora Xizangica, 5 vols.. Science Press, Beijing (in Chinese).

Xu, X.G., Wang, W., Wagner, M., Heussner, K.-U., Tarasov, P.E., Griess, B., Niu, S.S., Song, J.N., Xiao, Y.M., Cai, L.H., 2003. Results of the first archaeological excavation and environmental survey at Fengtai, Qinghai Province, PR China, in 2001. Eurasia Antiqua 9, 85–111.

Yan, G., Wang, F.B., Shi, G.R., Li, S.F., 1999. Palynological and stable isotopic study of palaeoenvironmental changes on the northeastern Tibetan plateau in the last 30,000 years. Palaeogeography, Palaeoclimatology, Palaeoecology 153, 147–159.

Yang, B., Bräuning, A., Dong, Z.B., Zhang, Z.Y., Jiao, K.Q., 2008. Late Holocene monsoonal temperate glacier fluctuations on the Tibetan Plateau. Global and Planetary Change 60, 126–140.

Yu, G., Tang, L.Y., Yang, X.D., Ke, X.K., Harrison, S.P., 2001. Modern pollen samples from alpine vegetation on the Tibetan Plateau. Global Ecology and Biogeography 10, 503–519.

Zhao, Y., Yu, Z.C., Chen, F.H., An, C.B., 2007. Holocene vegetation and climate changes from fossil pollen records in arid and semi-arid China. Developments in Quaternary Sciences 9, 51–65.

Zhu, L.P., Wu, Y.H., Wang, J.B., Lin, X.A., Ju, J.T., Xie, M.P., Li, M.H., Mäusbacher, R., Schwalb, A., Daut, G., 2008. Environmental changes since 8.4 ka reflected in the lacustrine core sediments from Nam Co, central Tibetan Plateau, China. The Holocene 18, 831–839.

Paper III

New Phytologist

Research

Tree endurance on the Tibetan Plateau marks the world's highest known tree line of the Last Glacial Maximum

Lars Opgenoorth[1,2], Giovanni G. Vendramin[3,*], Kangshan Mao[4,*], Georg Miehe[2], Sabine Miehe[2], Sascha Liepelt[1], Jianquan Liu[4] and Birgit Ziegenhagen[1]

[1]Faculty of Biology, Conservation Biology, University of Marburg, Karl-von-Frisch Strasse 8, 35032 Marburg, Germany; [2]Faculty of Geography, University of Marburg, Deutschhausstrasse 10, 35032 Marburg, Germany; [3]Istituto di Genetica Vegetale, Sezione di Firenze, Consiglio Nazionale delle Ricerche, Via Madonna del Piano, 50019 Sesto Fiorentino (FI), Italy; [4]Key Laboratory of Arid and Grassland Ecology, Institute of Molecular Ecology, Lanzhou University, Lanzhou 730000, China

Author for correspondence:
Lars Opgenoorth
Tel: 04964212822080
Email: Lars.Opgenoorth@staff.uni-marburg.de
Jianquan Liu
Tel: 00869318914305
Email: liujq@nwipb.ac.cn

*Authors who contributed equally to the work presented in this article.

Received: *28 April 2009*
Accepted: *12 July 2009*

New Phytologist (2009)
doi: 10.1111/j.1469-8137.2009.03007.x

Key words: glacial refugia, high-mountain areas, *Juniperus tibetica* complex, orographic leading edge, orographic rear edge, Tibetan Plateau.

Summary

- Because of heterogeneous topographies, high-mountain areas could harbor a significant pool of cryptic forest refugia (glacial microrefugia unrecognized by palaeodata), which, as a result of poor accessibility, have been largely overlooked. The juniper forests of the southern Tibetan Plateau, with one of the highest tree lines worldwide, are ideal for assessing the potential of high-mountain areas to harbor glacial refugia.
- Genetic evidence for Last Glacial Maximum (LGM) endurance of these microrefugia is presented using paternally inherited chloroplast markers. Five-hundred and ninety individuals from 102 populations of the *Juniperus tibetica* complex were sequenced at three polymorphic chloroplast regions.
- Significant interpopulation differentiation and phylogeographic structure were detected ($G_{ST} = 0.49$, $N_{ST} = 0.72$, $N_{ST} > G_{ST}$, $P < 0.01$), indicating limited among-population gene flow. Of 62 haplotypes recovered, 40 were restricted to single populations. These private haplotypes and overall degrees of diversity were evenly spread among plateau and edge populations, strongly supporting the existence of LGM microrefugia throughout the present distribution range, partly well above 3500 m.
- These results mark the highest LGM tree lines known, illustrating the potential significance of high-mountain areas for glacial refugia. Furthermore, as the close vicinity of orographic rear-edge and leading-edge populations potentially allows gene flow, surviving populations could preserve the complete spectrum of rear-edge and leading-edge adaptations.

Introduction

Based on pollen records, it is generally accepted that temperate and boreal forests of the world alternately became extinct at their poleward edges and expanded from their cold-stage macrorefugia following the climatic oscillations of the Pleistocene (Huntley & Birks, 1983; Bennett *et al.*, 1991; Lang, 1994; Hewitt, 1999; Prentice & Jolly, 2000; Frenzel *et al.*, 2003). However, macrofossil analyses, as well as genetic studies worldwide, are raising doubts about these glacial 'tabula rasa' scenarios for the leading edges (term 'leading edge' used *sensu* Hewitt, 2000). They indicate that pollen records are prone to 'overlook' cryptic refugia (from here on called microrefugia *sensu* Rull, 2009) of small sizes and of very local distribution (Willis *et al.*, 2000; Stewart & Lister, 2001; Willis & van Andel, 2004; Anderson *et al.*, 2006; Shepherd *et al.*, 2007; Pruett & Winker, 2008; Birks & Willis, 2009). Despite this growing evidence, the importance of microrefugia for preserving genetic diversity and speeding up Holocene recolonization is still widely underappreciated and interpretations of genetic data too often follow the dictate of pollen data, even when the coverage of pollen records is limited (Provan & Bennett, 2008). This is especially true for high-mountain areas that, because of their heterogeneous topography, should be ideal places for refugia and *in situ* persistence (Hewitt, 2004; Hampe & Petit, 2005) but owing to a lack of sufficient palaeorecords are often overlooked in this context. To demonstrate the potential importance of high-mountain areas for glacial forest endurances we present this case study of the forest history of the southern Tibetan Plateau using genetic markers to test for two possible hypotheses: glacial extinction with postglacial recolonization vs endurance of fragments of an interstadial forest. Both scenarios should provide distinctive genetic patterns: low degrees of genetic diversity within and between populations in the case of a recent recolonization from the plateau edges; and low within-population diversity but high diversity between populations as a result of genetic drift under *in situ* survival.

The Tibetan Plateau is the highest mountain plateau on earth, with 1.9 million km^2 being higher than 4 km above sea level (asl). It is largely covered by alpine pastures and alpine desert-steppe. Forests are limited to the eastern and southern declivities, with only some forest islands occurring on the plateau platform. Most forest stands are composed of *Juniperus*, *Betula* and *Picea* in the northeast (Qinghai province) and of *Juniperus* and *Betula* in the southern part of the Tibetan Plateau. Charcoals and macrofossils indicate a historically larger range of the juniper forests (Kaiser *et al.*, 2006, 2007, 2009; Miehe *et al.*, 2006, 2008). These forest remains have been interpreted as signs of a fragmented Holocene forest belt marginalized by anthropogenic influence as well as desiccation since the mid-Holocene climatic optimum (Miehe *et al.*, 2008; Kaiser *et al.*, 2009). On the basis of the few pollen records available, the existence of these forest islands has been attributed to Holocene recolonizations out of eastern and southeastern lowland refugia (Tang & Shen, 1996).

For the northeastern part of the Tibetan Plateau, this hypothesis has been supported by a phylogeographic study using chloroplast DNA (cpDNA) markers to analyze the genetic structure of *Juniperus przewalskii* forest islands. Six haplotypes were detected whose spatial distribution supports a Holocene recolonization from the eastern declivity (Zhang *et al.*, 2005). Also in the region, a similar pattern was shown for *Picea crassifolia* (Meng *et al.*, 2007).

In the present study, the analysis of forest history was extended to the southern part of the Tibetan Plateau by surveying the historical population dynamics of juniper forests. For this region, Frenzel *et al.* (2003) propose several glacial forest refugia in the deep valley gorges of the Mekong, Salween, Yangtze and Huang He (Fig. 1a). Because of the limited pollen data they draw support from indirect measures linking observed cold and drought limits of the present day's juniper forest distribution with paleoclimatological reconstructions. Based on these reconstructions they suggest an upper tree line between 3450 and 3600 m asl during the Last Glacial Maximum (LGM) (Frenzel *et al.*, 2003). Meanwhile, new records of extant juniper forests were found on the Tibetan Plateau at 4900 m asl, 200 m above the formerly known tree line (Miehe *et al.*, 2007). This extends the known limits of cold tolerance in this species. Following Frenzel's line of argument (Frenzel *et al.*, 2003), the potential LGM upper tree line would increase by 200 m to 3650–3800 m asl and therefore the area of potential LGM forest would increase significantly. Accordingly, and given that the idea of a large Tibetan Plateau ice shield has been soundly rejected (see Seong *et al.*, 2008), large parts of the southern Tibetan Plateau along the Yarlung Zhangbo catchment and the Kyi Chu River up to the city of Lhasa could have potentially supported forest growth during the LGM, thus including valleys throughout the present entire distribution range.

There are two main difficulties encountered when attempting to perform a phylogeographic study of the juniper forests in this region. First, the possibility of obtaining samples is limited because of the remoteness of the few remaining juniper stands. Second, these stands are made up of several tree species whose taxonomic classification is difficult and often arbitrary (Adams, 2004; Farjon, 2005). A morphological screening of several thousand specimens from these species covering their entire distribution range points to incomplete lineage sorting, or massive hybridization with species-specific characters unclearly delimited (L. Opgenoorth, G. Miehe & J. Liu, unpublished). Initial tests based on nuclear microsattelites (SSRs). SSRs also suggest strong hybridization (L. Opgenoorth, unpublished). Because focusing on a single species was thus infeasible, we decided to study a whole complex of closely related, interbreeding tree species, including *Juniperus tibetica*, *Juniperus indica*, *Juniperus convallium*, *Juniperus microsperma* and *Juniperus saltuaria* (Farjon, 2005). According to nuclear ITS Internal transcribed spacer and cpDNA sequences, this group (referred to as the *J. tibetica* complex hereafter) is monophyletic (Adams *et al.*, 2008). As the main focus of this study was not the analysis of introgression and hybridization processes, but the analysis of the large-scale spatial genetic structure of this group of species, we confined the analysis to uniparentally inherited cpDNA markers for inferring glacial refugia. More specifically, we wanted to: establish chloroplast genetic lineages within this complex; outline the phylogeographic and demographic history of each genetic lineage by means of genetic structure and allele frequencies; test the hypothesis of LGM forest endurance on the Tibetan Plateau based on genetic signatures; and integrate the molecular results with other palaeoecological evidence.

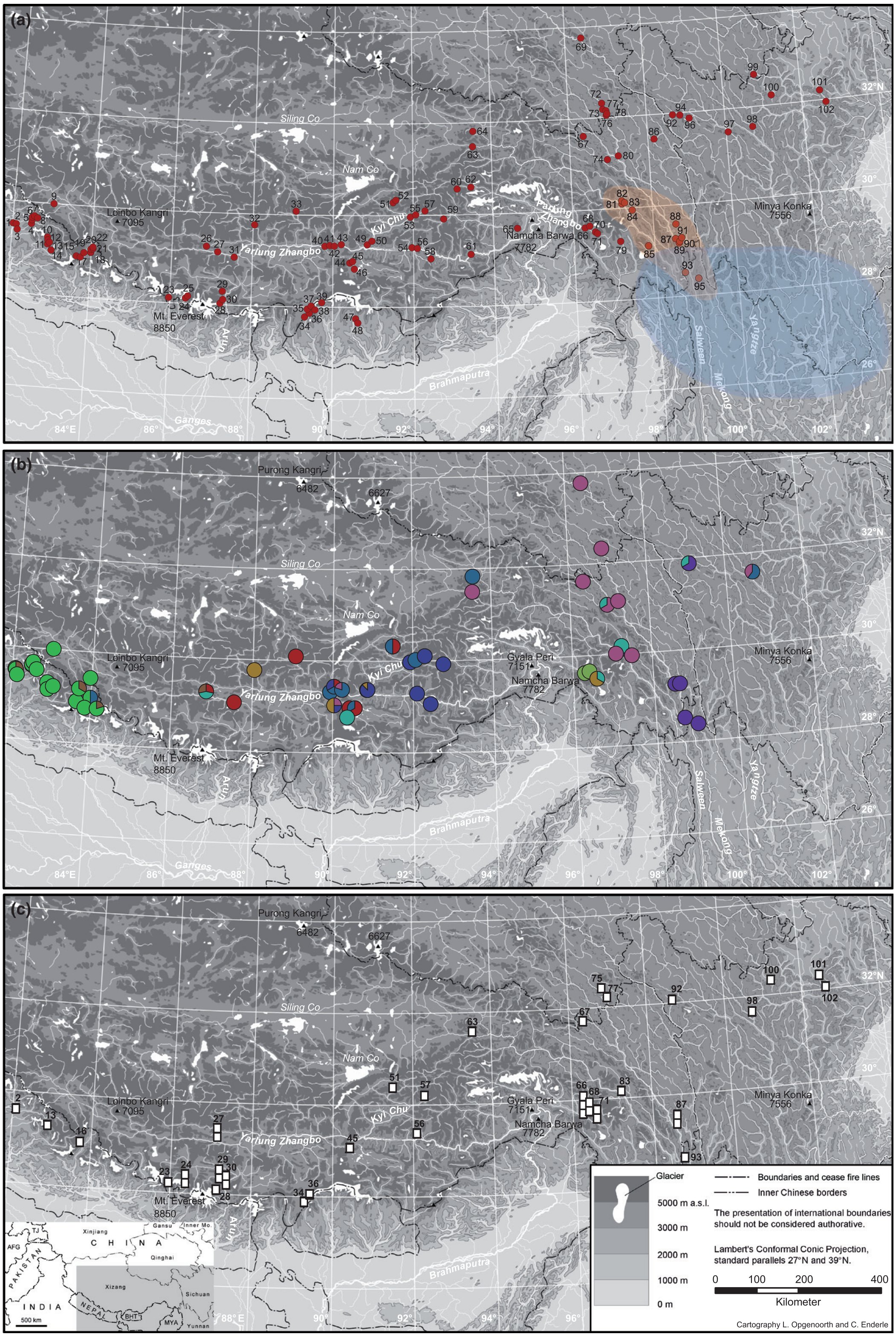


Fig. 1 (a) Map of 102 juniper populations. Orange shading reflects refugia proposed in Frenzel *et al.* (2003). Blue shading reflects a general southeastern refugium proposed by Tang & Shen (1996). (b) Regional distribution pattern of medium frequent haplotypes (8–100 individuals per haplotype). Each color reflects one haplotype. (c) Distribution of private haplotypes. Each column refers to one population. Each box in a column refers to a single private haplotype.

Materials and Methods

According to previous studies on other Cupressaceae species (Neale *et al.*, 1989, 1991; Mogensen, 1996; Kondo *et al.*, 1998; Hwang *et al.*, 2003), both cpDNA and mitochondrial DNA (mtDNA) are generally paternally inherited in this family. We therefore restricted our study of organelle DNA variation to the more polymorphic cpDNA (Petit & Vendramin, 2007). To identify potential cpDNA markers associated with glacial refugia, we first sequenced several noncoding cpDNA regions on a subset of the samples. This initial screening revealed three regions displaying sufficient polyDnaSPmorphism: the *trn*T-*trn*L Intergenic spacer IGS; the *trn*L-*trn*F IGS; and the *trn*L intron. Because these three regions are linked as a result of the uniparental inheritance of chloroplasts, they were combined to derive haplotypes. In total, 102 populations with a total of 590 specimens were sampled within the *J. tibetica* complex with five closely related putative species, namely *J. convallium*, *J. indica*, *J. microsperma*, *J. saltuaria* and *J. tibetica.* The sampled populations covered the whole distribution range of this hybrid complex (Fig. 1a; Table S1). Initial screening of 15 populations with > 10 individuals each showed that an increase in population number, rather than of individuals within populations, allowed detection of the highest haplotypic variation. Thus, for the remaining populations, five to nine samples were taken when available. For 46 sites, only one to four individuals were available because the samples were taken either from herbarium material (most of the Himalayan populations) or from populations of very limited size (one or a few individuals). The populations with one or two individuals were excluded from the statistical analyses.

Total DNA was extracted from leaves, as described by Dumolin *et al.* (1995). PCRs were performed using the primers and protocols described by Taberlet *et al.* (1991). Sequencing reactions were performed using the DYEnamic ET Dye Terminator Cycle Sequencing Kit (GE Healthcare, Munich, Germany) and run on a 96-capillary automated sequencer (MegaBACE 1000; GE Healthcare) following the manufacturer's protocols. Chromatograms were checked using the CHROMAS software (TECHNELYSIUM Pty Ltd, Tewantin, Qld, AU) and sequences were manually edited using CODONCODE v. 1.6.3 (CodonCode Corporation, 2007). Sequences were aligned using the CLUSTAL_X1.83 algorithm (Thompson *et al.*, 1994), as incorporated into CodonCode v. 1.6.3 (CodonCode Corporation, 2007), and corrected manually. They were assigned to different haplotypes using DnaSP 4.20 (Rozas *et al.*, 2003).

Genetic diversity and phylogeographic structure

Different glacial histories have been reported to cause varying patterns of genetic diversity within species. Usually, refugial populations show higher genetic diversity than postglacially established populations, except for situations where several recolonization lineages merge in a postglacially recolonized area (Hewitt, 2000; Petit *et al.*, 2003). However, as merging lineages blur phylogeographic structure, the latter case can be precluded in the event that phylogeographic structure is detected. In order to assess these scenarios, various measures of genetic diversity were estimated and the presence of a phylogeographic structure was tested.

First, allelic richness was estimated, after rarefaction for each population containing more than five samples, using Contrib 1.01 (Petit *et al.*, 1998). In order to test for decreasing diversity along a potential recolonization route, a Spearman rank correlation test between genetic diversity (theta pi obtained with Arlequin 3.11 Schneider *et al.*, 2000) and longitude, and between nucleotide diversity (π, following Tajima 1983, and Nei 1987, as implemented in Arlequin 3.11) and longitude was performed among plateau populations.

The existence of a phylogeographic structure was tested following Pons & Petit (1995, 1996) by calculating two measures of genetic differentiation: G_{ST} and N_{ST}. While G_{ST} is a differentiation measure that is based on allele frequencies only, N_{ST} takes into account the similarities between haplotypes (i.e. the number of mutations between haplotypes). Thus, while high G_{ST} values indicate a general geographic structure in the data, higher N_{ST} values than of G_{ST} values indicate phylogeographic structure, with closely related haplotypes being more likely to co-occur close to each other. Therefore, the two parameters were compared using a permutation test with 10 000 permutations and the *U*-statistic, as implemented in PERMUT (http://www.pierroton.inra.fr/genetics/labo/Software/Permut).

Phylogenetic relationships among haplotypes were inferred with MRBAYES 3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) using an F81+I+G substitution model following Mr.Modeltest2.2 (Nylander, 2004). Indels were treated as a separate block, using p-distances after simple coding (Simmons & Ochoterena, 2000) as implemented in seqstate v.1.32 (Müller, 2005). Bayesian analyses were conducted using two independent Markov Chain Monte Carlo runs with 10 million generations each, sampling trees every 1000 generations and with a burn-in of 1 million generations. One additional specimen (*Juniperus communis*, accession no. GQ301207) was included as the outgroup.

The presence of genetic barriers among populations was tested using the Monmonier maximum-differences algorithm implemented in the BARRIER 2.2 software (Manni *et al.*, 2004). The strength of this method is that it identifies geographic boundaries of abrupt change in genetic differences between pairs of populations based on a network obtained by Delaunay triangulation. Thus, population genetic structure can be analyzed spatially. As this approach requires a priori definition of the number (K) of groups, we ran BARRIER successively on our data set with increasing K until group structures started to dissolve to single populations. The genetic differentiation matrix was obtained using MEGA (Tamura *et al.*, 2007) and was based on p-distances to be able to include indel information.

Inference of demographic processes

Fu's F_S, as implemented in Arlequin 3.11 (Schneider *et al.*, 2000), and Fu and Li's D^* and F^* statistics, as implemented in DnaSP 4.50 (Rozas *et al.*, 2003), were used to test for deviations from neutrality.

In an attempt to further infer demographic processes, a mismatch distribution analysis was carried out using Arlequin 3.11 (Schneider *et al.*, 2000). The shape of the graph of the mismatch distribution is expected to be multimodal in samples drawn from populations at demographic equilibrium, whereas unimodal distributions are generally found in populations that have passed through a recent demographic expansion (Rogers & Harpending, 1992; Harpending *et al.*, 1998). It is important to stress that the shape of the distribution could potentially also be generated by a bottleneck event, and the distinction between the two demographic processes is difficult (Rogers & Harpending, 1992). The fit of the mismatch distribution to Poisson distributions was assessed by Monte Carlo simulations of 1000 random samples. The sum of squared deviations (SSDs) and raggedness (r) indices between observed and expected mismatch distributions were used as test statistics; their P-values represented the probability of obtaining a simulated SSD that was larger than or equal to the observed SSD.

Finally, separate haplotype networks for each genetic lineage, revealed by the phylogenetic analysis, were constructed using TCS 1.21 (Clement *et al.*, 2000) to obtain additional information about past demographic history of the species. Network ambiguities were resolved following the criteria suggested by Crandall & Templeton (1993): (1) rare haplotypes are more likely to be found at the tip, and common haplotypes found at interior nodes of a cladogram; (2) a singleton (i.e. a haplotype represented by a single individual) is more likely to be connected to haplotypes from the same population than to haplotypes from different populations.

Results

Genetic diversity

Sequencing of the *trn*T-*trn*L IGS, the *trn*L-*trn*F IGS and the *trn*L intron resolved 28 single nucleotide polymorphisms and 16 indels with lengths varying from 1 to 82 bp. These resulted in 62 different haplotypes with sizes ranging from 922 to 1014 bp and an alignment length of 1109 bp (accession no.: GQ268173–GQ268222, GQ285817–GQ285847).

Haplotype frequencies are reported in Table S2. Most of the haplotypes were rare haplotypes with 33 singletons and five haplotypes recorded only twice. The two most frequent haplotypes (H13, H25) accounted for 45% of the samples, and the six most frequent haplotypes accounted for 71% of the samples. Accordingly, while the majority of haplotypes (40) was fixed in one population (Fig. 1c), the three most frequent haplotypes occurred in populations throughout the plateau. Haplotypes of medium frequency generally showed regional distribution patterns (Fig. 1b).

The number of haplotypes per population ranged between 1 and 6 (allelic richness after standardization to a common size of five, using the rarefaction method, ranged between 1 and 4.5, Table S1). Most populations showed little variation. Of the 73 populations with $n \geq 3$, 16 displayed one haplotype, 27 displayed two haplotypes and 22 displayed three haplotypes. Only two populations (P71 and P30) contained six and five haplotypes, respectively. No clear geographic pattern could be observed in the distribution of the populations displaying the highest variation. This can be seen in Fig. 2a, which shows populations with above- and below-average levels of richness. Likewise, the private haplotypes (haplotypes fixed in that population) were evenly spread all over the distribution range (Fig. 1c).

The Spearman rank correlation test between genetic diversity (theta pi) and longitude and between nucleotide diversity (π) and longitude among plateau populations showed no significant correlation (rs (Spearman's rho) = 0.03, P = 0.185; and rs = 0.03, P = 0.179, respectively).

Phylogeographic structure

A strong signal for phylogeographic structure was found. The N_{ST} (0.72) was significantly larger than the G_{ST} (0.49, $P < 0.01$), demonstrating that gene-flow is low relative to mutation rate. Three genetic lineages with distinct distributions were identified (Fig. 3). Genetic lineage 1 (GL1) included haplotypes H1–H4, H16 and H17. Genetic lineage 2 (GL2) comprised haplotypes H35–H39 and H46–H59. These two genetic lineages were distributed in specific geographic areas (Fig. 2b). Genetic lineage 1 comprised populations 66 and 68 in the Parlung Zhangbo valley (Fig. 2b). Genetic lineage 2 was almost exclusively confined to the Himalayan populations, with the exception of H57 and H55 that occurred also in two plateau populations (P9 and P26, respectively) (Fig. 2b). Furthermore, GL2 was subdivided into two subgroups (called GL2a and GL2b Fig. 3), which correspond to separate geographic regions (Fig. 2b).

Genetic lineage 3 (GL3) included all remaining haplotypes, for example, those having no sufficient posterior probabilities and those consisting of too few haplotypes and

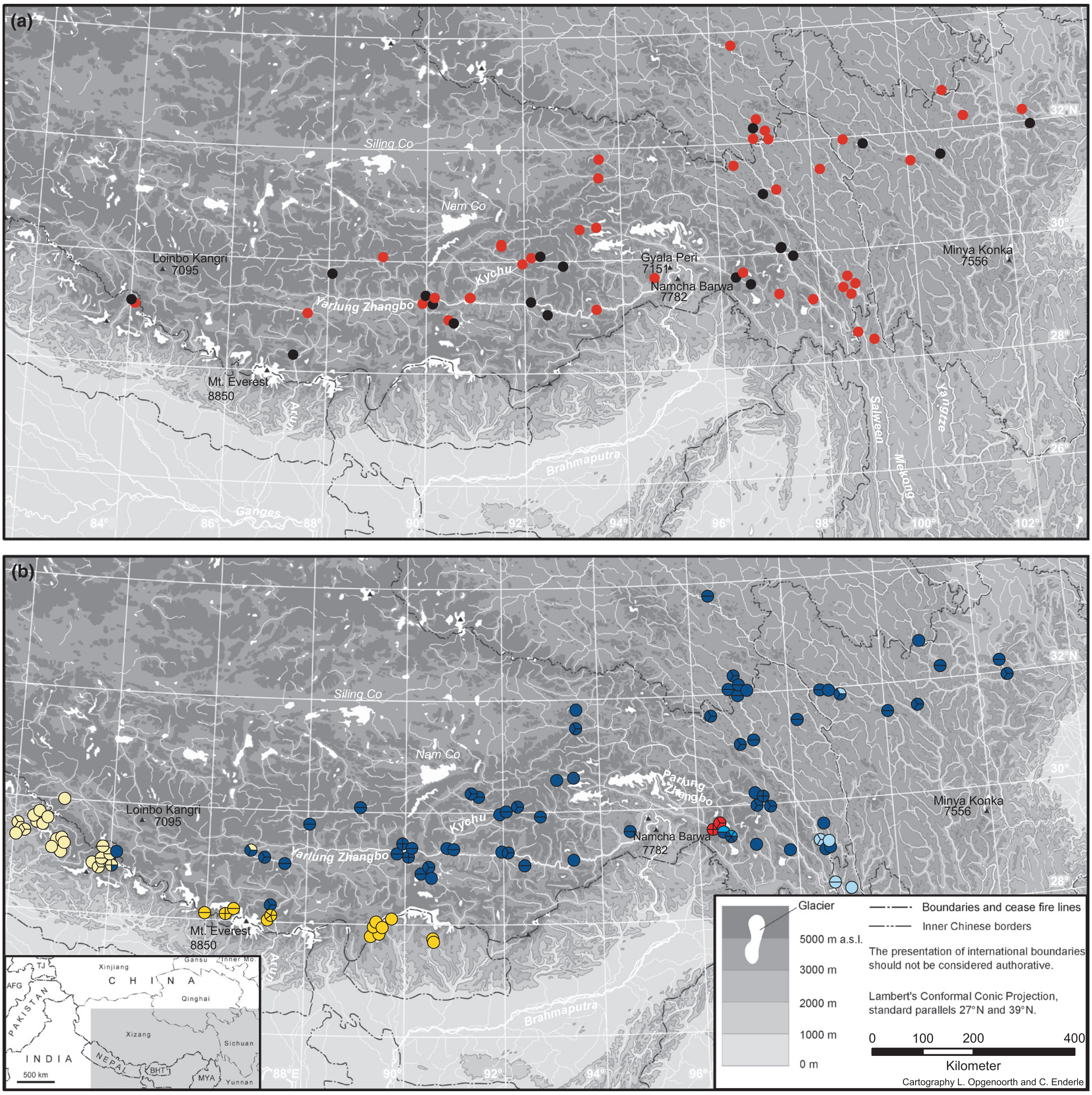


Fig. 2 (a) Juniper populations of above-average (black) and below-average (red) allelic richness. (b) Populations according to genetic lineages (GLs). GL1, red; GL2a, light yellow; GL2b, dark yellow; GL3a, light blue; GL3b, medium blue; GL3c, dark blue. Boxes refer to single haplotypes.

too few splits with just one bifurcation to legitimate separate lineages (H18–H20 and H5–H8; Fig. 3). Nevertheless, both subgroups H18–H20 and H5–H8 are geographically confined to limited areas, thus also contributing to the phylogeographic structure (Fig. 2b).

Two striking disjunctions were observed in GL3, with haplotype H26 occurring in the Himalayan population P20 and in the easternmost plateau population P102, and haplotype H43 was found only in the Himalayan population P17.

Monmonier's maximum difference algorithm confirmed the groups derived by the phylogeny, with a split between the Himalayan populations and the plateau populations, a differentiation of the two Himalayan groups and the split of GL1 from the rest (figure not shown). It did not resolve the split between the GL3 subgroups, however. Additional increase in the number of *K* led to a dissolution of group structure by singling out individual populations with endemic haplotypes.

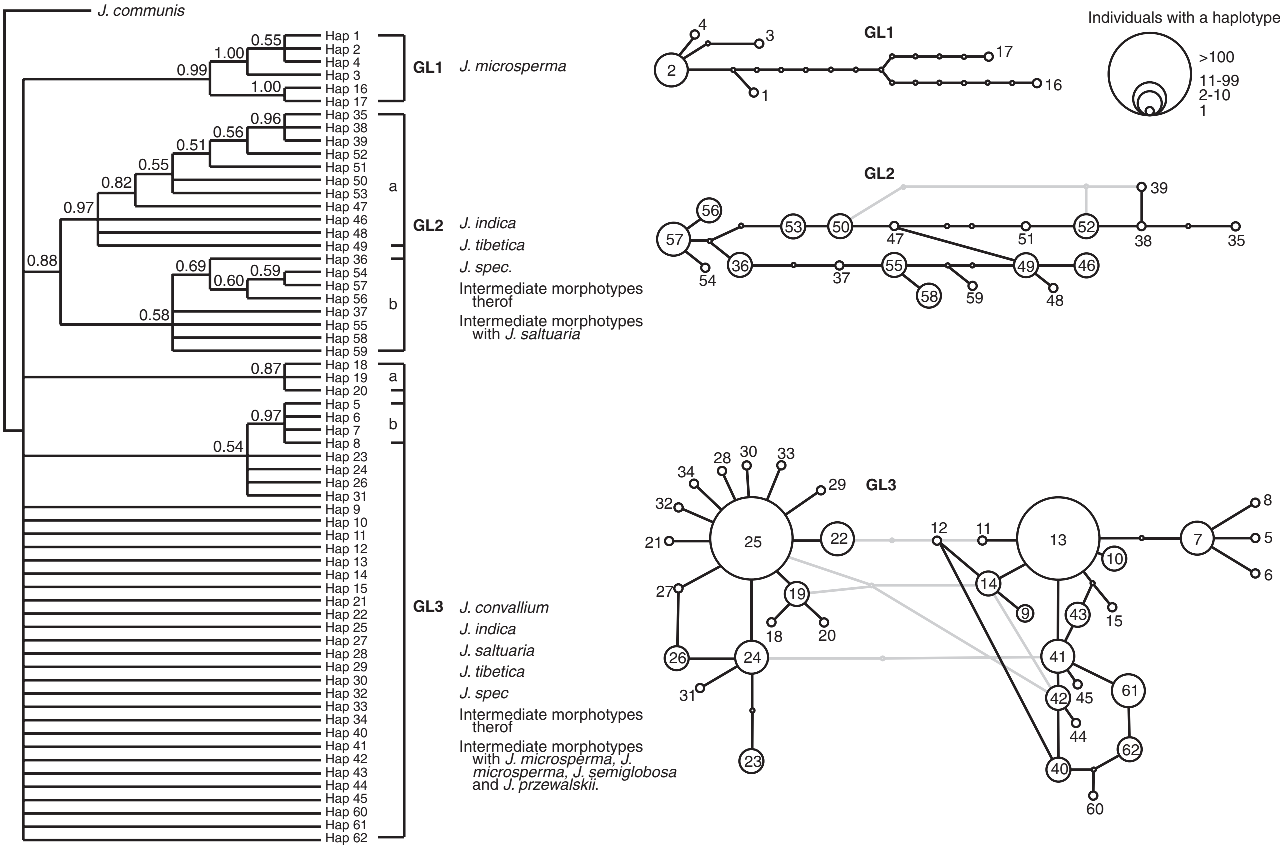


Fig. 3 Phylogeny with genetic lineages (GLs) and putative morphological species with haplotype networks of the respective genetic lineages.

Neutrality tests and demographic processes

Fu's *Fs* statistics produced large negative values for GL2 and GL3, showing deviances from neutrality (Table 1). As Fu and Li's *D* and *F* statistics were both not significant and background selections were thus unlikely causes for the deviance from neutrality, historic population growths or severe bottlenecks remained two possible explanations for the observed patterns (Fu, 1997). For GL1, all neutrality test statistics for deviation from neutrality were nonsignificant (Table 1).

The mismatch distributions for all genetic lineages were unimodal (figures not shown), with the SSD values between the observed and the expected mismatches and Harpending's *r* indices being not significant (Table 1), confirming the neutrality test results for GL2 and GL3 and contradicting the results for GL1.

The minimum spanning network for GL1 showed a star-like pattern (Fig. 3) with one dominating haplotype (H2 comprising 19 of the 24 samples) and five singletons. A star-like pattern is considered to indicate a demographic expansion (Hudson, 1990), thus supporting the findings of the mismatch distribution.

As a result of its complex topography, the GL2 haplotype network gave no clear indication regarding demographic processes (Fig. 3). The uneven sampling in the Himalayan populations could potentially have distorted the frequencies in the minimum spanning network.

Because many haplotypes differed by only one or a few mutations, the GL3 minimum spanning network produced closed loops that could not be resolved in all cases. Nevertheless, the most abundant haplotypes were interior

Table 1 Demographic expansion

GL	SSD	*P*-value	HRI	*P*-value	*Fs*	*P*-value	*D**	*P*-value	*F**	*P*-value
GL1	0.042	0.16	0.348	0.43	0.720	0.67	−1.57	>0.10	−1.97	>0.10
GL2	0.006	0.63	0.018	0.73	−26.0	0.00	−0.61	>0.10	−0.61	>0.10
GL3	0.023	0.06	0.064	0.13	−26.5	0.00	−2.05	>0.05	−1.79	>0.10

*D**, Fu and Li's *D** test statistic; *F**, Fu and Li's *F** test statistic; *Fs*, Fu's *Fs* test statistic; GL, genetic lineage; HRI, Harpending's raggedness index; SSD, sum of squared deviation under expansion model.

haplotypes, and most of the singletons were tip haplotypes (Fig. 3), as would be expected under coalescent theory (Hudson, 1990). The overall network is as complex as the GL2 but it comprised some clades with star-like patterns, hinting at demographic expansions within some of the subclades.

Discussion

The classical glacial 'tabula rasa' scenario for forests on the Tibetan Plateau assumes the complete extinction of the former interstadial forests and postglacial recolonization from southern and eastern macrorefugia located in the deep valley gorges (Tang & Shen, 1996). Accordingly, as observed for *J. przewalskii* in Qinghai (Zhang *et al.*, 2005), all haplotypes recorded at present would have dispersed from these refugia or would have appeared during the potential range expansion. The high amount and even distribution of private haplotypes throughout the range of the *J. tibetica* complex provides a very strong argument against this scenario. As dispersal of private haplotypes to single populations throughout the range is very unlikely, these private haplotypes would have had to evolve within a time span of approx. 14 000 yr or less following the LGM. Even though exact mutation rates are not available for the chloroplast sequences employed in this study, the information available for cpDNA in general, as well as results with the same sequences reported in other tree species (Anderson *et al.*, 2006; Magri *et al.*, 2007), clearly contradict this hypothesis. For example, Graur & Li (2000) report an average mutation rate of $1.2–1.7 \times 10^{-9}$ substitutions per site per year for cpDNA. Given that the average sequence length in this study is 1000 bp, a single mutation in one of those sequences should occur every 580 000–1 000 000 yr. Thus, even if all 62 haplotypes detected in this study would differ by only one mutation to all other haplotypes it would be highly unlikely that all of these mutations occurred within the past 20 000 yr. Instead, there are several haplotypes, even from the western margin of the plateau, that carry two substitutions compared with their closest recorded relative (e.g. H23 and H60), thus increasing the time span to 1 060 000–2 000 000 yr or even longer when considering the closest plateau edge haplotype. Furthermore, if these mutations had occurred during such a short time span, one would expect higher overall haplotypic diversity, in particular in refuge populations, considering that they had at least an order of magnitude more time for haplotype accumulation.

Instead, a large proportion of the populations are fixed for one or two haplotypes regardless of their geographic location. Furthermore, overall degrees of diversity are not significantly different between the plateau populations and the Himalayan populations. This also holds true when comparing the diversity of the plateau populations of this study with that of plateau edge populations for *J. przewalskii* in the northeast (Zhang *et al.*, 2005). Additionally, if all plateau populations had been extirpated during the LGM, successive founder events during the recolonization would have led to a decline of intrapopulation diversity along the recolonization route. This phenomenon has been frequently observed in the European biota along a south–north gradient (Hewitt, 1996; Comps *et al.*, 2001). As we could show that the Himalayan populations did not contribute to the current populations of the plateau, a potential recolonization would have had to follow an east–west route and thus a decline should be recognizable along that route. This could not be found among the juniper populations analyzed in this study.

A corroborative line of argument against the notion of postglacial recolonization relies on the finding of distinct regional geographic patterns observed with haplotypes of intermediate abundance (Fig. 1b). Except for the haplotypes from GL1 that are linked to *J. microsperma*, the distinct regional patterns do not reflect species boundaries but instead seem to be largely independent from the taxonomic identity of the samples. Thus, such a spatial genetic pattern can either be attributed to populations recently founded by long-distance dispersal events during recolonization (leptokurtic dispersal) (Hewitt, 1993; Ibrahim *et al.*, 1996; Bialozyt *et al.*, 2006), or they can be explained by demographic re-expansion of previously present populations that had recently experienced fragmentation accompanied by bottleneck events and genetic drift. The strong phylogeographic structure indicates that gene flow among the Tibetan Plateau junipers has been severely limited, at least since the formation of the observed geographic/genetic pattern. High G_{ST} values could potentially reflect vegetative reproduction, although this phenomenon has never been reported for these species nor has it been observed in any of the > 100 populations screened for this research. Nevertheless, because long-distance dispersal events tend to minimize phylogeographic structure (Petit *et al.*, 2004), they do not seem to have played a significant role in juniper dispersal. This also has implications for interpreting the geographical haplotype disjunctions identified. Such disjunctions can be attributed to homoplasy, long-distance dispersal or to the fragmentation of formerly more widespread haplotypes. As homoplasy is unusual in the conservative chloroplast genome and long-distance dispersal has also been shown to be an unlikely contributor, the presence of these disjunctions also seems to contradict postglacial recolonizations and to suggest that the fragmentation of formerly more widespread haplotypes is involved.

Finally, the strong phylogeographic structure demonstrates that the Himalayan populations generally did not contribute to the current colonization of the plateau, even though they were much closer to the upper Yarlung Zhangbo and the Kyi Chu catchment populations than a potential southeastern refugium. This pattern does not seem plausible under a postglacial recolonization scenario that is dependent on high postglacial migration rates. Again, a model of ancient fragmented forest patches appears to explain these results more parsimoniously.

The genetic data presented here strongly suggest that the juniper forest islands and isolated tree stands of the southern Tibetan Plateau are remnants of a former interstadial forest that were fragmented during the last LGM and that experienced postglacial local expansions before again experiencing fragmentation and marginalization as a result of anthropogenic influence as well as desiccation. In addition, we speculate that the clear separation of the Himalayan haplotypes reflected a much older haplotype-distribution pattern, possibly dating back to stages of the uplift of the Himalayas and the plateau during the late Tertiary. Similar cases have recently been made for *Quercus suber* in the Mediterranean basin (Hampe & Petit, 2007; Magri *et al.*, 2007) where the pattern was correlated with the break up and separation of several microplates during the Miocene, and for *Quercus lobata* in California, whose genetic structure was found to 'most likely reflect[s] the impact of the Tertiary' (Grivet *et al.*, 2006). Interestingly, the clear split between Himalayan haplotypes and plateau haplotypes did not strictly follow the orographic barriers, as Himalayan haplotypes 'leaked' onto the plateau at the western margin of the Kyi Chu watershed, while plateau haplotypes reached 'Himalayan territory' in the Arun watershed (Fig. 2b), diminishing the role of the mountain range as a migration barrier.

These results clearly contradict the former perception of forest glacial history on the southern Tibetan Plateau and the phylogeographic results reported for *J. przewalskii* on the northeastern Tibetan Plateau (Zhang *et al.*, 2005). The different fate of the plateau platform populations of *J. przewalskii* in the northeast and the species reported on in this study can probably be attributed to the topographical and ecological differences of these regions. Most notably, the intersecting valleys of the large rivers lead to a larger altitudinal amplitude of approx. 400 m in the distribution range of southern plateau platform populations, in contrast to the distribution range of northeastern plateau populations of *J. przewalskii.* This larger altitudinal amplitude resulted in a larger climatic buffer of > 2° (0.55°/100 m Bohner, 2006) for southern tree populations during the LGM. Moreover, while the altitudes increase gradually from edges to platform on the northeastern Tibetan Plateau, the altitudinal amplitudes of the river valleys are contained and thus provide niches throughout the southern Tibetan Plateau, especially considering that relatively steep southerly exposed valley slopes would receive additional solar heat.

As the juniper tree species can be considered keystone species on the Tibetan Plateau, these findings considerably alter the preconditions for understanding the glacial history of other plant and animal species in the region. Likewise, the ongoing discussion on whether humans inhabited the plateau throughout the LGM or whether they recolonized the plateau at the onset of the Holocene (Aldenderfer, 2006) can profit from these findings as even small forests or woodlands could have provided humans with additional essential resources.

On a global scale our findings stress the potential for microrefugia in the patchy landscapes of high-mountain areas with their availability of diverse environments. This is emphasized by the fact that the valley bottoms of the refugia presented here largely exceed 3500 m, and partly even 4000 m, asl and thus mark the highest LGM tree lines known in the world so far. Furthermore, our results stress the importance of small surviving populations for a species' demographic and evolutionary history. This is even more so when considering the adaptive potential of microrefugia in high mountain areas where strong elevational gradients have a similar effect on populations as have leading and stable rear edges ('stable rear edge' *sensu* Hampe & Petit, 2005) in latitudinal ranges by maintaining adaptive potential for heat and drought tolerance at low altitude (or at the stable rear edge) and cold tolerance and dispersal ability at high altitude (or at the leading edge) (Hampe & Petit, 2005). However, by contrast to the latitudinal rear and leading edge populations, orographic rear and leading edges are in close vicinity to each other, potentially allowing for gene flow. We thus propose that refugia in the orographic stable rear edges could even harbor both adaptive potentials, by bridging the gap between leading and rear edges and thus increasing their evolutionary importance.

In conclusion, the ecological and evolutionary significance of high-mountain areas needs serious re-appraisal, both in terms of conservation efforts as well as in understanding the evolutionary history of species. In addition, the ever-increasing number of microrefugia detected provokes re-evaluation of the importance and speed of postglacial migration processes and population dynamics. In turn, prediction on the impact of future climate change on such dynamics will also have to change.

We hope that this study will encourage further studies aiming to identify and analyze microrefugia, especially in high-mountain areas, to scrutinize forest histories and to assess the adaptive potential of such populations.

Acknowledgements

The authors thank the members of the Lhasa University Expedition Team 2004 for assistance with field data collection. Rémy Petit and Martin Lascoux, as well as three anonymous reviewers, provided critical comments on an earlier version of the manuscript. This research was supported by grants from the German Research Council (DFG Grants Mi 271-18 and Zi 698-6), the German Academic Exchange

Program (DAAD) and by grants from the National Natural Science Foundation of China (30430560, 30725004) to J.Q.L. The original manuscript was linguistically improved by Anita Hopes.

References

Adams RP. 2004. *Junipers of the world: the genus Juniperus.* Vancouver, Canada: Trafford Publishing.

Adams RP, Morris JA, Schwarzbach AE. 2008. Taxonomic affinity of rushforth's Bhutan juniper and *Juniperus indica* using SNP's from nrDNA and cp trnC-trnD, terpenoids and RAPD data. *Phytologia* **90**: 233–245.

Aldenderfer M. 2006. Modelling plateau peoples: the early human use of the world's high plateaux. *World Archaeology* **38**: 357–370.

Anderson LL, Hu FS, Nelson DM, Petit RJ, Paige KN. 2006. Ice-age endurance: DNA evidence of a white spruce refugium in Alaska. *Proceedings of the National Academy of Sciences, USA* **103**: 12447–12450.

Bennett KD, Tzedakis PC, Willis KJ. 1991. Quaternary refugia of north European trees. *Journal of Biogeography* **18**: 103–115.

Bialozyt R, Ziegenhagen B, Petit RJ. 2006. Contrasting effects of long distance seed dispersal on genetic diversity during range expansion. *Journal of Evolutionary Biology* **19**: 12–20.

Birks JB, Willis K. 2009. Alpines, trees, and refugia in Europe. *Plant Ecology & Diversity* **1**: 147–160.

Bohner J. 2006. General climatic controls and topoclimatic variations in Central and High Asia. *Boreas* **35**: 279–295.

Clement M, Posada D, Crandall KA. 2000. TCS. A computer program to estimate gene genealogies. *Molecular Ecology* **9**: 1657–1659.

CodonCode Corporation 2007. Codoncode Aligner [2.0]. Computer program. CodonCode Corporation Dedham, MA, USA.

Comps B, Gomory D, Letouzey J, Thiebaut B, Petit RJ. 2001. Diverging trends between heterozygosity and allelic richness during postglacial colonization in the European beech. *Genetics* **157**: 389–397.

Crandall KA, Templeton AR. 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* **134**: 959–969.

Dumolin S, Demesure B, Petit RJ. 1995. Inheritance of chloroplast and mitochondrial genomes in pedunculate oak investigated with an efficient PCR method. *Theoretical and Applied Genetics* **91**: 1253–1256.

Farjon A. 2005. *A monograph of Cupressaceae and Sciadopitys.* Kew, UK: Royal Botanic Gardens.

Frenzel B, Bräuning A, Adamczyk S. 2003. On the problem of possible last-glacial forest-refuge areas within the deep valleys of Eastern Tibet. *Erdkunde* **57**: 182–198.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147**: 915–925.

Graur D, Li W-H 2000. *Fundamentals of molecular evolution.* Sunderland, MA, USA: Sinauer Associates, Inc., Publishers.

Grivet D, Deguilloux MF, Petit RJ, Sork VL. 2006. Contrasting patterns of historical colonization in white oaks (*Quercus* spp.) in California and Europe. *Molecular Ecology* **15**: 4085–4093.

Hampe A, Petit RJ. 2005. Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters* **8**: 461–467.

Hampe A, Petit RJ. 2007. Ever deeper phylogeographies: trees retain the genetic imprint of Tertiary plate tectonics. *Molecular Ecology* **16**: 5113–5114.

Harpending HC, Batzer MA, Gurven M, Jorde LB, Rogers AR, Sherry ST. 1998. Genetic traces of ancient demography. *Proceedings of the National Academy of Sciences, USA* **95**: 1961–1967.

Hewitt GM. 1993. Postglacial distribution and species substructure: lessons from pollen, insects and hybrid zones. In: Lees DR, Edwards D, eds. *Evolutionary patterns and processes. Linnean Society symposium series 14.* London, UK: Academic, 97–123.

Hewitt GM. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58**: 247–276.

Hewitt GM. 1999. Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* **68**: 87–112.

Hewitt G. 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907–913.

Hewitt GM. 2004. Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **359**: 183–195.

Hudson RR. 1990. Gene genealogies and the coalescent process. In: Futuyma D, Antonovics J, eds. *Oxford surveys in evolutionary biology.* Oxford, UK: Oxford University Press, 1–44.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

Huntley B, Birks HJB. 1983. *An atlas of past and present pollen maps for Europe: 0-13000 Years Ago.* Cambridge, UK: Cambridge University Press.

Hwang SY, Lin TP, Ma CS, Lin CL, Chung JD, Yang JC. 2003. Post-glacial population growth of *Cunninghamia konishii* (Cupressaceae) inferred from phylogeographical and mismatch analysis of chloroplast DNA variation. *Molecular Ecology* **12**: 2689–2695.

Ibrahim KM, Nichols RA, Hewitt GM. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity* **77**: 282–291.

Kaiser K, Miehe G, Schoch WH, Zander A, Schlütz F. 2006. Relief, soil and lost forests: Late Holocene environmental changes in southern Tibet under human impact. *Zeitschrift für Geomorphologie, Supplement* **142**: 149–173.

Kaiser K, Schoch WH, Miehe G. 2007. Holocene paleosols and colluvial sediments in Northeast Tibet (Qinghai Province, China): Properties, dating and paleoenvironmental implications. *CATENA* **69**: 91–102.

Kaiser K, Opgenoorth L, Schoch WH, Miehe G. 2009. Charcoal and fossil wood from palaeosols, sediments and artificial structures indicating Late Holocene woodland decline in southern Tibet (China). *Quaternary Science Reviews* **28**: 1539–1554.

Kondo T, Tsumura Y, Kawahara T, Okamura M. 1998. Paternal inheritance of chloroplast and mitochondrial DNA in interspecific hybrids of *Chamaecyparis* spp. *Breeding Science* **48**: 177–179.

Lang G 1994. *Quartäre Vegetationsgeschichte Europas. Methoden und Ergebnisse.* Jena, Germany: Fischer.

Magri D, Fineschi S, Bellarosa R, Buonamici A, Sebastiani F, Schirone B, Simeone MC, Vendramin GG. 2007. The distribution of *Quercus suber* chloroplast haplotypes matches the palaeogeographical history of the western Mediterranean. *Molecular Ecology* **16**: 5259–5266.

Manni F, Guerard E, Heyer E. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. *Human Biology* **76**: 173–190.

Meng LH, Yang R, Abbott RJ, Miehe G, Hu TH, Liu JQ. 2007. Mitochondrial and chloroplast phylogeography of *Picea crassifolia* Kom. (Pinaceae) in the Qinghai-Tibetan Plateau and adjacent highlands. *Molecular Ecology* **16**: 4128–4137.

Miehe G, Miehe S, Schlutz F, Kaiser K, Duo L. 2006. Palaeoecological and experimental evidence of former forests and woodlands in the treeless desert pastures of Southern Tibet (Lhasa, AR Xizang, China). *Palaeogeography Palaeoclimatology Palaeoecology* **242**: 54–67.

Miehe G, Miehe S, Vogel J, Co S, Duo L. 2007. Highest treeline in the northern hemisphere found in southern Tibet. *Mountain Research and Development* **27**: 169–173.

Miehe G, Miehe S, Will M, Opgenoorth L, Duo L, Dorgeh T, Liu JQ. 2008. An inventory of forest relicts in the pastures of Southern Tibet (Xizang AR, China). *Plant Ecology* **194**: 157–177.

Mogensen HL. 1996. The hows and whys of cytoplasmic inheritance in seed plants. *American Journal of Botany* **83**: 383–404.

Müller K. 2005. SeqState – primer design and sequence statistics for phylogenetic DNA data sets. *Applied Bioinformatics* **4**: 65–69.

Neale DB, Marshall KA, Sederoff RR. 1989. Chloroplast and mitochondrial-DNA are paternally inherited in *Sequoia sempervirens* D. Don Endl. *Proceedings of the National Academy of Sciences, USA* **86**: 9347–9349.

Neale DB, Marshall KA, Harry DE. 1991. Inheritance of chloroplast and mitochondrial-DNA in incense-cedar (*Calocedrus decurrens*). *Canadian Journal of Forest Research-Revue Canadienne de Recherche Forestiere* **21**: 717–720.

Nei M. 1987. *Molecular Evolutionary Genetics.* Columbia University Press, New York, NY, USA.

Nylander JAA. 2004. *MrModeltest v.2.* Uppsala, Sweden: Uppsala University.

Petit RJ, Vendramin GG. 2007. Plant phylogeography based on organelle genes: an introduction. In: Weiss S, Ferrand N, eds.*Phylogeography of Southern European Refugia – evolutionary perspective on the origins and conservation of European biodiversity.* Springer, 23–97.

Petit RJ, El Mousadik A, Pons O. 1998. Identifying populations for conservation on the basis of genetic markers. *Conservation Biology* **12**: 844–855.

Petit RJ, Aguinagalde I, de Beaulieu JL, Bittkau C, Brewer S, Cheddadi R, Ennos R, Fineschi S, Grivet D, Lascoux M *et al.* 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. *Science* **300**: 1563–1565.

Petit RJ, Bodenes C, Ducousso A, Roussel G, Kremer A. 2004. Hybridization as a mechanism of invasion in oaks. *New Phytologist* **161**: 151–164.

Pons O, Petit RJ. 1995. Estimation, variance and optimal sampling of gene diversity .1. Haploid locus. *Theoretical and Applied Genetics* **90**: 462–470.

Pons O, Petit RJ. 1996. Measuring and testing genetic differentiation with ordered versus unordered alleles. *Genetics* **144**: 1237–1245.

Prentice IC, Jolly D. 2000. Mid-Holocene and glacial-maximum vegetation geography of the northern continents and Africa. *Journal of Biogeography* **27**: 507–519.

Provan J, Bennett KD. 2008. Phylogeographic insights into cryptic glacial refugia. *Trends in Ecology & Evolution* **23**: 564–571.

Pruett CL, Winker K. 2008. Evidence for cryptic northern refugia among high- and temperate-latitude species in Beringia – a response to Stewart and Dalen (2008). *Climatic Change* **86**: 23–27.

Rogers AR, Harpending H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution* **9**: 552–569.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* **19**: 2496–2497.

Rull V. 2009. Microrefugia. *Journal of Biogeography* **36**: 481–484.

Schneider S, Roessli D, Excoffier L 2000. *Arlequin 3.11: a software for population genetics data analysis.* Geneva: Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.

Seong YB, Owen LA, Bishop MP, Bush A, Clendon P, Copland L, Finkel R, Kamp U, Shroder JF. 2008. Quaternary glacier history of the Central Karakoram – reply. *Quaternary Science Reviews* **27**: 1656–1658.

Shepherd LD, Perrie LR, Brownsey PJ. 2007. Fire and ice: volcanic and glacial impacts on the phylogeography of the New Zealand forest fern *Asplenium hookerianum. Molecular Ecology* **16**: 4536–4549.

Simmons MP, Ochoterena H. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* **49**: 369–381.

Stewart JR, Lister AM. 2001. Cryptic northern refugia and the origins of the modern biota. *Trends in Ecology & Evolution* **16**: 608–613.

Taberlet P, Ludvic G, Guy P, Jean B. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology (Historical Archive)* **17**: 1105–1109.

Tajima F. 1993. Evolutionary relationship of DNA sequences in finite populations. Genetics **105**: 437–460.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**: 1596–1599.

Tang LY, Shen CM. 1996. Late Cenozoic vegetational history and climatic characteristics of Qinghai-Xizang Plateau. *Acta Micropalaeontologica Sinica* **13**: 321–337.

Thompson JD, Higgins DG, Gibson TJ. 1994. Clustal-W – improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**: 4673–4680.

Willis KJ, van Andel TH. 2004. Trees or no trees? The environments of central and eastern Europe during the Last Glaciation. *Quaternary Science Reviews* **23**: 2369–2387.

Willis KJ, Rudner E, Sumegi P. 2000. The full-glacial forests of central and southeastern Europe. *Quaternary Research* **53**: 203–213.

Zhang Q, Chiang TY, George M, Liu JQ, Abbott RJ. 2005. Phylogeography of the Qinghai-Tibetan Plateau endemic *Juniperus przewalskii* (Cupressaceae) inferred from chloroplast DNA sequence variation. *Molecular Ecology* **14**: 3513–3524.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Population description and their diversity measures

Table S2 Distribution of haplotypes among populations

Paper IV

Conservation Genet Resour
DOI 10.1007/s12686-009-9062-3

TECHNICAL NOTE

Identification and characterization of microsatellite marker in the tetraploid *Juniperus tibetica* Kom. using next generation sequencing

Lars Opgenoorth

Received: 10 July 2009 / Accepted: 25 July 2009

Abstract *Juniperus tibetica* Kom. is a tetraploid endemic tree species of the southern Tibetan Plateau. For this species ten microsatellite markers were developed using next generation sequencing. To characterize these loci a sample of 14 juniper individuals from seven populations covering large parts of the species' distribution range was analyzed. All loci were polymorphic, ranging from two to 15 alleles per locus. Expected heterozygosity was estimated using the program TETRASAT, and ranged from 0.20 to 0.90 across loci. These markers will be used in ongoing population genetic studies to evaluate the potential genetic depauperation of the highly fragmented forest remains on the southern Tibetan Plateau and to propose strategies for conservation management.

Keywords *Juniperus tibetica* · Microsatellites · Tibetan Plateau · Tetraploid

Juniperus tibetica Kom. (Cupressaceae) is a tetraploid endemic taxon on the southern Tibetan Plateau and its adjacent declivities (Farjon 2005). As one of the last remaining forest tree species on the Tibetan Plateau it is a keystone species in the area (Miehe et al. 2008). A recent phylogeographic study using cpDNA sequence variation showed that *J. tibetica* and four closely related species have survived the last glacial maximum on the Tibetan Plateau (Opgenoorth et al. accepted) in decentralized microrefugia. The study furthermore implies that these populations have been relatively stable probably since the uplift of the Plateau but at least throughout the Pleistocene. The strong fragmentation and little abundance of these forests make them one of the most threatened elements of the current landscape in High Asia. Programs for maintaining its genetic resources and genetic diversity should be established. Therefore, knowledge is needed about nuclear genetic diversity and the amount of recurrent gene flow. To be able to perform these analyses we developed and here present such markers for ongoing population genetic research in *Juniperus tibetica*.

Total genomic DNA of one *Juniperus tibetica* tree was extracted from leave tissue following the protocol by Dumolin (Dumolin et al. 1995). The DNA was subjected to shotgun sequencing on the GS-FLX at Duke IGSP Sequencing Core Facility using GS FLX Titanium reagents. 1/8 of a plate produced 67.781 reads with a total of 22.9 Mb and an average read lengths of 339 bases and a mode at 419 bases. The reads were combined in a single FASTA file and screened for mono-, di-, tri-, tetra, penta- and hexanucleotide repeats using MSATCOMMANDER (Faircloth 2008). The number of minimum repeats was set to 12, 10, 7, 6, 5, and 4 respectively. Primers were designed with the workflow inbuilt in MSATCOMMANDER which includes primer 3 (Rozen and Skaletsky 2000). With this pipeline 272 primer pairs were retrieved. However, many of them were omitted immediately since they or the microsatellite motifs they amplify revealed non-desired properties like extremely too long and compound-interrupted simple sequence repeat (SSR) stretches, SSR stretches too close to the vector, too short stretches, or in few cases, because the original sequence and thus microsatellite motif was homologous to another sequence. From the remaining primer pairs 50 were chosen, and ordered from METABION, Martinsried, Germany. To cost-efficiently test this high number of loci the fluorescent labeling was done according to the procedure described by Schuelke (Schuelke 2000).

L. Opgenoorth (✉)
Conservation Biology, University of Marburg, Karl-von-Frisch Strasse 8, 35032 Marburg, Germany
e-mail: Lars.Opgenoorth@staff.uni-marburg.de

Following an extensive prescreening for the quality of the amplified SSRs, which was performed on a subset of four individuals run on an automatic capillary sequencer (MegaBACE 1000, Amersham Bioscience), ten single loci SSRs were found to have scorable polymorphic bands (accession numbers…). The other 40 loci showed no amplification, multibanding patterns, too pronounced stutter and/or monomorphic bands, respectively. To characterize the ten confirmed loci, a total of 14 individuals were sampled from seven different populations from large parts of the species' distribution range.

DNA was isolated from needles as mentioned before. PCRs were performed in 25 µl containing 30 ng of template DNA, 1× PCR buffer (Promega), 5 m of each dNTPs, 1 U *Taq* polymerase (Go *Taq*, Promega), 1.5 m and 1.5 m $MgCl_2$ respectively (Table 1), 0.5% BSA and 2 µm of each primer. Two different PCR profiles were used to amplify the microsatellite loci: (a) denaturation at 95°C for 5 min followed by 30 cycles at 94°C (30 s), respective annealing temperature (see Table 1), 72°C (45 s), followed by eight cycles 95°C (30 s), 53°C (45 s), 72°C (45 s), and a final extension at 72°C for 10 min; and (b) denaturation at 95°C for 5 min, 10 touchdown cycles at 95°C (30 s), 60°C (30 s) (−1°C/cycle), 72°C (40 s), 25 cycles at 94°C (30 s), 50°C (30 s), 72°C (40 s), and final extension at 72°C 7 min. PCRs were performed using a Biometra T1 thermo cycler (Whatman Biometra, Goettingen, Germany). The amplification products were separated by capillary electrophoresis using MegaBACE 1000 (Amersham Bioscience) automatic sequencer. Alleles were sized using the size standard MegaBACE ET400-R (Amersham Bioscience) and the MegaBACE Fragment Profiler version 1.2 software (Amersham Bioscience). Primer pairs are reported in Table 1.

Due to the tetraploidy of *Juniperus tibetica* exact allele frequencies could not be determined. As a consequence a test for linkage disequilibrium was also not possible due to the unknown allele dosage of partial heterozygotes. However, expected Hardy–Weinberg heterozygosity (H_E) was calculated using the software TETRASAT, which computes all possible allele combinations for partial heterozygotes and reports a mean value for H_E (Markwith et al. 2006). The derived H_E ranged from 0.20 to 0.90 across the loci (see Table 1). As the samples tested do not derive from one single outcrossing population, the values of expected heterozygosity have to be treated with reserve. However, the high number of alleles detected at the ten SSR loci promises a sufficient power for population-level analysis of genetic diversity, and genetic structure.

In the nearest future, the described microsatellite markers will be used to analyze the genetic structure and diversity of juniper as affected by landscape fragmentation on the southern Tibetan Plateau. Furthermore, research on effective gene flow and spatial analyses will be performed.

Table 1 Characterization of 10 microsatellite loci

Locus	GenBank accession no.	Repeat motif	Primer sequence forward	Primer sequence reverse	Tm	Size range (bp)	No. of alleles	H_E	SD
JT01		CTT	TTGTCTCCCCTGCACACTC	GCAGGCAGCCATGAGAAAG	56°	176–221	8	0.79	0.01
JT02		ACAT	GCAATGGTAGATACTTGGGATTCAG	TGGAATATGTATGCAGCTAGGTC	62.5°	156–248	11	0.84	0.01
JT03		AAC	AGTCGAGGAAACAACTACAATCC	TTGTTGGGCGCATTTTGTC	51°	169–193	7	0.73	0.02
JT04		AAG	ATTCAAGGGATGAGCACAAAG	CAGGCTAATCCACACACTTCAC	62.5°	183–216	3	0.55	0.03
JT05		AACAAT	ACAATGTTGGTCCCTACAACAAC	AGGAACACTTCCATTATTTGGATAGG	TD	203–209	2	0.20	0.03
JT06		AAAATG	CCTCCGCTCTTGTCGCAG	GATTTGTATTGCTCAAATCCTTCAG	TD	243–283	4	0.71	0.01
JT07		AG	TTGGGTTCAACGGTTTGCC	CCATTCACGGCCTACTCAC	TD	213–243	12	0.86	0.01
JT08		CT	CGGGTGAATCAAGCGGAAATC	CCAATGGAAACCATAACAAACACC	TD	239–251	7	0.77	0.02
JT09		AT	ACAAAGAACAAGCAATGGAATACTG	TGGGACTCAACCACATGGC	55°	174–233	13	0.90	0.00
JT10		AG	TCGCGAGTAAGACCATGAGG	ACTCACTTGGAGCATAACATAAC	62.5°	131–213	5	0.73	0.02

H_E, expected heterozygosity and *SD* standard deviation following computations for tetraploid organisms by the software TETRASAT. *TD* touch down program as specified in the text

Acknowledgments I would like to thank Tina Mengel for providing essential assistance with the lab work and Giovanni Vendramin and Sascha Liepelt for their valuable advice. Furthermore I would like to thank Lisa Bukovnik and the Duke IGSP Sequencing Core Facility for their service. This research was supported by grants from the German Research Council (DFG Grants Mi 271-18 and Zi 698-6), and the German Academic Exchange Program (DAAD).

References

Dumolin S, Demesure B, Petit RJ (1995) Inheritance of chloroplast and mitochondrial genomes in pedunculate oak investigated with an efficient PCR method. Theor Appl Genet 91:1253–1256

Faircloth BC (2008) MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. Mol Ecol Resour 8:92–94

Farjon A (2005) A monograph of cupressaceae and sciadopitys. Royal Botanic Gardens, Kew

Markwith SH, Stewart DJ, Dyer JL (2006) TETRASAT: a program for the population analysis of allotetraploid microsatellite data. Mol Ecol Notes 6:586–589

Miehe G, Miehe S, Will M, Opgenoorth L, Duo L, Dorgeh T, Liu JQ (2008) An inventory of forest relicts in the pastures of Southern Tibet (Xizang AR, China). Plant Ecol 194:157–177

Opgenoorth L, Vendramin GG, Mao K, Miehe G, Miehe S, Liepelt S, Liu JQ, Ziegenhagen B (accepted) Tree endurance on the Tibetan Plateau marks the world's highest known tree line of the last glacial maximum. New Phytol

Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 132:365–386

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233–234

Research Proposal V

VolkswagenStiftung

Antragstellung in der Förderinitiative

„EVOLUTIONSBIOLOGIE“

Bitte beachten Sie bei der Antragstellung die Kriterien des entsprechenden Merkblattes für Antragsteller.

Bitte laden Sie sich dieses Formular herunter, füllen Sie es aus und schicken Sie es als Attachment (unverschlüsselt) per E-Mail zurück an:

hartmann@volkswagenstiftung.de

Hinweis zum Datenschutz
Wir weisen darauf hin, dass die VolkswagenStiftung in Übereinstimmung mit datenschutzrechtlichen Vorschriften Ihre personenbezogenen Angaben ebenso speichert wie alle weiteren Antragsdaten.

Antragsdarstellung (insgesamt ca. 8 Seiten) fontsize 11 pt. in englischer Sprache

Titel *(klar formuliertes und aussagefähiges Thema) in deutscher und englischer Sprache*

„Adaptation at the limits” or “the limits of Adaptation” - Tracing abiotic stress-tolerance in Tibetan juniper trees along altitudinal and ecological gradients.

Zusammenfassung *in deutscher und englischer Sprache* ***(ca. 150 Worte)***
Im Bewilligungsfall wird diese Zusammenfassung einige Zeit später im Internet aufgeführt.

High mountain areas like the Tibetan Plateau exhibit a range of extreme ecological gradients on a relatively small spatial scale. This potentially leaves specific imprints on the genetic makeup and the distribution of adaptive diversity among plant and animal species. In order to trace adaptive traits related to drought, cold, and UV-tolerance this study uses High Throughput sequencing techniques in association mapping to study the juniper forests of the Tibetan Plateau. Once characterized, the candidate genes of these traits will be used to test key ecological hypothesis about diversity distribution among rear and leading edge populations, test for adaptive introgression, and compare genes essential to speciation with genes related to adaptation to the key stresses.

Hochgebirgsregionen wie das Tibetische Plateau sind charakterisiert durch extreme ökologische Gradienten in relative begrenzter räumlicher Distanz. Diese räumliche Struktur hinterlässt Abdrücke in der genetischen Zusammensetzung und der Verteilung adaptiv relevanter Diversität in Tieren und Pflanzen. In dieser Studie werden Hoch-Durchsatz Sequenziertechniken verwendet, um adaptiv relevante Merkmale im Zusammenhang mit Trocken-, Kälte- oder UV-Stress zu finden. Hierzu werden Assoziations-Studien an Tibetischen Wacholderwäldern durchgeführt. Sobald die Kandidatengene besagter Merkmale charakterisiert sind, werden sie dafür genutzt, ökologische Hypothesen bezüglich der Verteilung von adaptiv relevanter Diversität zwischen ‚rear edge’ und ‚leading edge’ Populationen zu testen. Darüber hinaus soll mit Hilfe der Kandidatengene geklärt werden, ob es zwischen den nahe verwandten Wacholderarten zu adaptiver Introgression kam. Schließlich sollen die in begleitenden Studien gefundene Gene zur Artunterscheidung mit den genannten adaptiv relevanten Genen verglichen werden, um zu überprüfen, ob Anpassung an Schlüsselstressoren zur Artbildung beigetragen hat.

Internationaler Stand der Forschung

Scientific Background

In the light of Global Change (GC) and with the advent of high throughput sequencing techniques, studies on the adaptive potential of plant and animal species to changing environments are an increasingly focal point of evolutionary biology. The aims of these studies are to characterize and understand ecologically important complex traits, identify and trace different forms of selection processes, and localize adaptive diversity within and among populations in order to mitigate potential risks of species extinctions and thus protect ecosystem functioning.

Despite their exceptional importance as key species in landscapes, trees have been dealt with comparatively little regarding the detection of complex traits due to the fact that as long-lived and sessile organisms they are usually considered difficult experimental organisms (Neale & Savolainen 2004). Additionally the genomes of tree species are often extremely large, especially those of conifers. Therefore sequencing essential parts of their genomes has been not feasible so far (Neale & Savolainen 2004).
On the other hand, a growing number of studies on economically important genera as *Pinus*, *Populus*, *Quercus,* and *Fagus* has emerged in recent years. These studies usually focus on traits such as wood quality, drought hardiness, fire-, and cold-tolerance either for economic reasons or as Climate Change (CC) will likely have major impacts on them (e.g. Eveno *et al.* 2008; Gonzalez-Martinez *et al.* 2006). In these studies population-level sequence information from nuclear candidate genes has proven useful for reconstructing historical demographic processes (e.g. Heuertz et al. 2006) for *Pinus sylvestris* and for identifying genes that have contributed to local adaptation (e.g. Eveno *et al*., 2008) for *Pinus pinaster*. Moreover, it has been shown that many modern trees display adaptive differentiation in relation to latitude or elevation (Sork *et al*. 1999). It is thus likely that in the close past, the interaction between selection and gene flow contributed to rapid adaptation of environmental sensitivness of populations throughout species range in conjunction with post-glacial migrations Davis & Shaw 2001. In this context the distinct position of rear edge populations has been stressed. Being generally small sized and over a long time isolated, these populations usually show reduced within-population genetic diversity but exceptionally high levels of differentiation among such populations. Furthermore, it was suggested that due to their isolation they have been subjected to selection for local adaptation rather than for vagility and generalism potentially leading to very distinct ecotypes (Review in Hampe & Petit 2005). This concept is largely based on results from neutral marker. A test with adaptive relevant genes is thus needed. Furthermore, this largely latitudinal

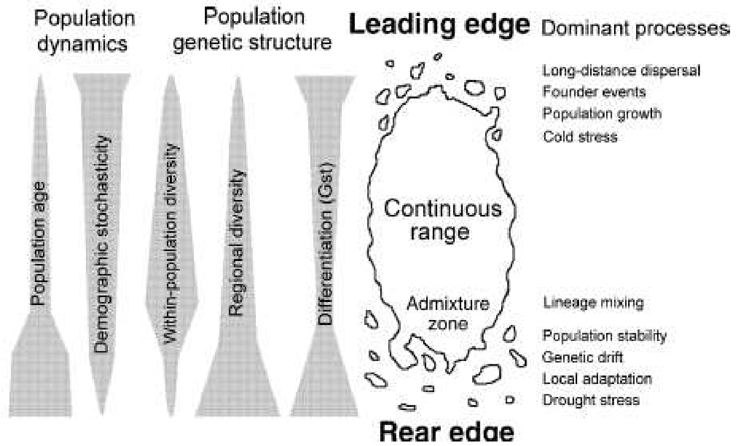


Figure 1 Population features and relevant processes at the leading and the rear edge of species ranges. (Hampe & Petit 2005).

concept has been modified for orographic situations in high mountain areas recently (Opgenoorth et al. subm.), where it was hypothesised that orographic rear edges (low elevational populations) should potentially harbour the adaptive potential of rear and leading edge since the close vicinity to orographic leading edges could potentially allow gene flow. A test with neutral marker is currently under way and should be followed by a test with adaptive marker.

In general, some characteristics of trees should play an important role to withstand environmental changes (Kramer *et al*., 2008): i) trees have high phenotypic plasticity that allows them to withstand large environmental fluctuations during their lifetime (Rehfeldt *et al*., 2002) ii) there are high levels of genetic diversity for allozymes and nuclear markers within – rather than between – populations (Hamrick 2004) iii) and gene flow – especially of pollen – occurs over large distances, thereby exchanging favourable genetic variants between isolated stands (Petit & Hampe 2006). In conclusion as trees are assumed to rely on standing genetic variation rather than on new mutations (Aitken *et al*. 2008) it has been hypothesised that the process of adaptation to new environmental conditions could be surprisingly swift (Davis *et al*. 2005; Petit *et al.* 2008). Furthermore, as hybridization is a common phenomenon among many tree taxa the potential of the standing genetic variation can in some cases be increased by a process called adaptive hybridization – or adaptive trait introgression, where adaptations of closely related species are incorporated into the genepool of a species through hybridization (e.g. Kim *et al.* 1999).

However, in areas where forest species approach the limits of trees' physiological amplitudes already today the potential of their standing genetic variation as well as that of related species can be assumed to be fully exploited. This should make them exceptionally suited to study adaptive traits. Furthermore, as population sizes there are usually small, heavily fragmented, and reproduction already constricted, additional stress due to climatic or other environmental stresses could overburden the phenotypic plasticity, thus increasing the potential for extinction (Davis & Shaw 2001; Jump & Penuelas, 2005) To be able to address and mitigate these challenges research is extremely needed.

The forests and woodlands of the southern and north-eastern Tibetan Plateau fit the outlined scenario as they withstand some of the harshest environmental conditions for forest growth on earth with the highest northern treeline at 4.900 m. a.s.l. (Miehe *et al* 2008) as well as drought limits for tree growth at around 260 mm/a (Miehe *et al.* 2008) and UV radiation among the highest in the world. Only few tree genera – (e.g. *Betula*, *Picea*, *Juniperus*) cope with the extreme gradients of drought, limitation in warmth and high UV radiation along latitudinal as well as elevational transects (Miehe *et al.* 2008). Despite (or because of) the extreme environmental conditions outlined above the genus *Juniperus* has a large centre of diversity in this environmental setting with a range of endemic and non-endemic, partly hybridizing, species occurring throughout the Plateau as well as parts of the adjacent Himalayan and Eastern declivities (Adams 2004). The radiation of this genus has been linked to the uplift of the Tibetan Plateau and the adjacent Mountain Ranges suggesting that either allopatric speciation took place due to emerging orographic barriers or adaptive speciation in the light of emerging environmental challenges. However, heavy hybridization has been observed among different juniper species potentially widening their respective ecological amplitude in the past.

Darstellung des Vorhabens mit
- **Zielsetzung**
- **Durchführung, Methoden**
- **Arbeits- und Zeitplan**

Aim of the study

The aim of this study is to assess the adaptive potential in key adaptive traits of three closely related, interbreeding juniper tree species of the Tibetan Plateau, namely *Juniperus tibetica*, *Juniperus saltuaria* and *Juniperus convallium*, as a model for a keystone tree species in a high mountain area. The originality of this proposal is that it aims to i) trace a magnitude of adaptive strategies due to the strong selection pressures of this extreme environment ii) get information on the adaptive potential and evolutionary history of key traits at the physiological limits of tree growth iii) use, for the first time, large scale adaptive genomics approach to test essential concepts of population genetics regarding the distribution and differentiation of adaptive potential of (orographic) rear and (orographic) leading edge populations iv) analyse the role of adaptive introgression /adaptive hybridization in tree species v) verify whether adaptive relevant genes differ from genes relevant to speciation. More specifically we intend to

i) Identify and characterize genes for drought-, cold- and UV-radiation-tolerance in *Juniperus tibetica, Juniperus saltuaria and Juniperus convallium*

ii) Identify polymorphisms at the selected candidate genes

iii) Compare the diversity of adaptive traits of orographic rear edge populations with orographic leading edge populations and with latitudinal rear edge populations

iv) Compare patterns of nucleotide diversity at candidate genes in the three species in allopatric and sympatric situations

v) Characterize genes relevant for speciation (performed by Dr. J. Liu based partly on his sequencing and on the pyrosequencing performed in this project)

Realization/ methods

The approach will be based on high-throughput (HT) sequencing of candidate genes and genotyping of SNPs at these candidate genes, and phenotyping of adaptive traits in native tree populations. The main limitation in such studies is not so much obtaining but handling the massive amounts of data. This research project can greatly profit from collaboration with partners within and associated to the Evoltree Network that provide bioinformatic pipelines (Michele Morgante, David Neale) to handle the amount of data. The main environmental drivers chosen for our project are drought, cold/limitation in warmth, and UV-radiation as these are the most pronounced environmental challenges in the research area and will be affected in future climate change scenarios. Furthermore cold and drought tolerance are associated with leading and rear edge populations respectively (see above), which are a specific target of this research.

The emerging method of choice for such studies is association mapping (Neale and Savolainen 2004). It combines genome-wide sampling with traditional population genetic approaches to understanding evolution (Gonzalez-Martinez *et al.*, 2006). A detailed review of how an integrated genomics approach can be conducted to identify patterns of adaptive variation in natural population can be found in Gonzalez-Martinez *et al* (Gonzalez-Martinez *et al.*, 2006). Briefly, alleles (haplotypes) at candidate genes for adaptive traits and their effects on phenotypes need to be characterized via sequencing and association mapping. At this stage, functional genomics can assist in understanding gene action and regulation by providing detailed transcriptional profiles. Second, frequencies of alleles in native populations for causative single-nucleotide polymorphisms are estimated to identify patterns of adaptive variation across heterogeneous environments.

A comprehensive list of candidate genes is available from INRA Bordeaux, David Neale and Giovanni Vendramin derived from physiological studies and other genomic studies. Still it is a key challenge of this project to characterize genes of adaptive traits as transferability among taxa is relatively low, in particular across species not belonging to the Pinaceae. Transferability tests including Tibetan junipers are being performed at the moment by Giovanni Vendramin. On the other hand it should be stressed that the key reference for this research will be setting up a EST database by pyrosequencing cDNA of the three juniper species by a third party. This approach should also allow the comparison of speciation genes vs. adaptation genes.

Large parts of the work requires sequence analysis and annotation using bioinformatics pipelines. Despite great advances with these pipelines, previous experience of large scale projects showed that manual control of the candidate genes after the automated handling is an important and, in some cases, a time consuming task (Gonzalez-Martinez & Vendramin pers. comm.). Reseqeuncing for SNP detection will be performed with a set of samples already available from former collections. The large scale sampling design will follow the outline by comparable studies from within Evoltree adjusted to the situation on the Tibetan Plateau. Briefly, the intention is to sequence between 100 and 140 genes per trait or SNP-genotype 384 SNP’s or a multiple of this number depending on the results of the first steps. The number of individuals per species will be a minimum of 95 distributed along 5-6 replicates for each elevational stage (12-24 individuals per replicate and species). The population sampling will reflect local environmental variation and will be based on knowledge from our preparatory work as well as from remote sensing and climate data obtained from Dr. Christoph Reudenbach, University of Marburg.

To be able to phenotype large numbers of individuals in the remote setting of the Tibetan Plateau, priority will be given to traits that can be measured in the labs after sample collection. Thus sampling and phenotyping field work will essentially be combined.

The genotyping platform will be set up in cooperation with the lab of David Neale. Also the bioinformatics pipeline from the Neale lab will be used for processing these data.

Screening and resequencing of genes impacting speciation is currently under way and will be concluded by our Partner Jianquan Liu. Adaptational, spatial and diversity analysis as well as publication of the results will be done in close cooperation between all partners.

Working plan

i) Pyrosequencing of cDNA from *J. tibetica, J. saltuaria, J. convallium* – 130.000 reads each.
ii) Use of bioinformatic pipeline developed within Evoltree to organize the species gene index for further molecular investigations, perform the functional annotation
iii) Identify genes of potential ecological importance related to drought-, cold-, and UV-tolerance. This task will greatly benefit from the cooperation within the Evoltree Network (www.evoltree.eu) as well as the cooperation with the CRSP project at UC Davis (www.dendrome.ucdavis.edu/crsp) that will make

available over 1,000 conifer genes that are currently tested for transferability to other conifer species, junipers included.

iv) Resequencing a set of ca. 100-140 candidate genes to enable the in silico mining of single nucleotide polymorphism (SNPs), using the sequencing platform of Neale Lab.

v) Establish permanent research plots in natural forests (in cooperation with Prof. Dr. Jianquan Liu, Lanzhou University and Dr. Georg Miehe, University of Marburg) along ecological and altitudinal gradients based on our knowledge of the existing forest islands in Tibet and based on climatological and remote sensing data (cooperation with Dr. Christoph Reudenbach, University of Marburg).

vi) Development of sequencing/genotyping platform

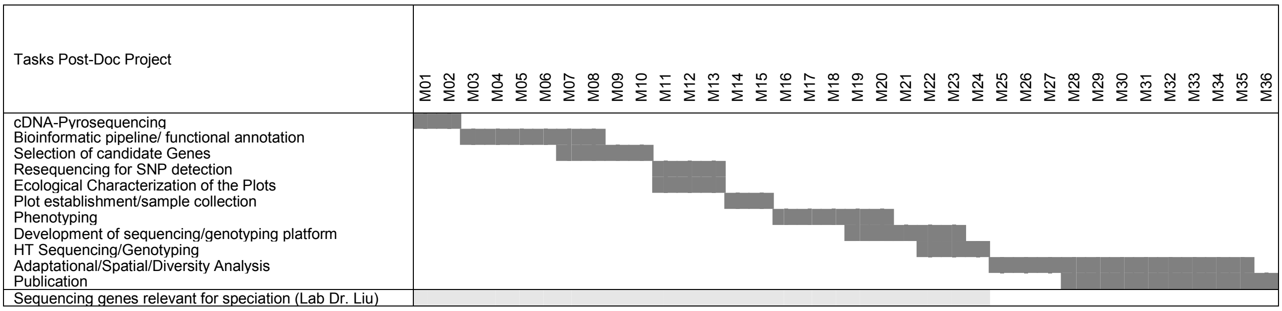
vii) HT Genotyping using the Illumina Platform

viii) Measure the impact of allelic polymorphism at candidate genes on phenotypes in the model forests along ecological gradients.

ix) Adaptational/Spatial/Diversity Analysis

x) Publications

Zeitplan



Eigene Vorarbeiten

Preparatory work

Lars Opgenoorth

1.) Phylogeography with cpDNA sequencing of a 100 populations of the hybrid complex throughout its distribution range. Important basis for choosing the three model species from the hybrid complex. Important for hypothesis building.
2.) Development of nSSRs for the *Juniperus tibetica* hybrid complex.
3.) Use of nuclear and chloroplast markers to assess recurrent gene flow/fragmentation.
4.) Use of marker to test for neutrality. Tests for neutrality are an important prerequisite for association mapping.
5.) Extensive collection of juniper samples (frozen at -80℃). This material can be used for immediately starting pyrosequencing.
6.) In collaboration with the working group of Prof. Dr. Miehe, Geography, University of Marburg, extensive biogeographic and ecological research on the juniper forests including macrofossil, charcoal, and pollen analysis, an inventory of the remaining juniper forests on the Tibetan Plateau and the adjacent Himalayas, and nursery trials since 1997 have been performed (since 2003 supported by VW-Foundation). This information is basis for choosing sample locations, for starting common garden experiments as well as the Light Chamber experiments, and for hypothesis building.
7.) Participation in researcher trainings within Evoltree and with David Neale’s Lab for population genomics and genomics of adaptation. One additional training will be attended in the near future for annotation procedures.

Giovanni G. Vendramin

1.) Test of candidate genes for drought resistance in different conifer species.
2.) Analysis of nucleotide diversity in conifer species. Test of linkage disequilibrium decay in conifers.
3.) Currently resequencing of hundreds of candidate genes in many conifer species including samples from Tibetan junipers is under way.
4.) Preparation of papers about nucleotide diversity in conifer species is in progress

David Neale

1.) Establishment of bioinformatics pipeline that can be used for this research proposal.
2.) Allocation of candidate genes developed for other conifer species.

Jianquan Liu

1.) Study on Phylogeny of Tibetan juniper species currently under way
2.) Extensive screening of genes relevant in speciation process currently also under way.

Christoph Reudenbach

1.) Climate and remote sensing of parts of the Tibetan Plateau. This will be basis for choosing the research sites.

Angaben zur

- **thematischen Ausrichtung des Gastlabors bzw. Gastinstitution**
- **Begründung der Wahl der Arbeitsgruppe / Universität**
- **Stellungnahme der Leitung der gastgebenden Arbeitsgruppe** (bitte als Anlage beifügen!)

Genexpress, CNR, Florence
Giovanni Vendramin and his lab have been working in forest genetics for over 15 years with an outstanding history in primer development, phylogeographic and population genetic studies. For the past 3 years they have been involved in forest genomics approaches with adaptive traits.

The lab of Giovanni Vendramin belongs to the leading forest genetic labs in the world as shown by his impressive list of over 150 papers in international journals and through his collaborations worldwide. The lab has tremendous experience both in project planning, realiziation and publication of studies. The project proposal has in large parts profited from his advice.

Neale lab, UC Davis
The primary research interest of this lab is the discovery and understanding of function of genes in forest trees, especially those controlling complex traits. They are focusing on SNP discovery within candidate genes and association mapping to identify alleles useful in tree breeding. A committment to developing tree breeding technologies will not only provide better wood and paper products, but will also reduce the need for harvesting of natural forests.

This lab can probably be considered the pioneering team of using genomic approaches for adaptation in trees. The bioinformatic pipeline developed at Neale's lab will be essential in this project. Furthermore, intellectual support and exchange will be a tremendous asses to this research as well as for my career.
I have met David and his team at a workshop they organised on adaptive genomics in trees.

Gerhard Müller-Starck, TU München
Gerhard Müller-Starck has been the leader of the department of forest genetics at the TU Munich for 14 years and has been involved in european forest genetics as for example in the Evoltree network. His lab is one of 4 labs in Germany dealing with forest genomics. Furthermore his working group is one of the first to use proteomics to trace stress-response in trees in collaboration with Dr. Dieter Ernst, from the Fraunhofer-Institute München.

I hope to profit from the great experience from Müller-Starcks lab within Forest genetics and forest genomics. Furthermore, as I hope to use this post-doc project as a platform for further collaborations I intend to raise funds for a PhD project that would implement a proteomics approach to test juniper's stress-response to UV-Radiation (see motivation letter). This project would greatly benefit from the know-how from Gerhard Müller-Starck and his team.

Matthias Fladung, BFH Großhansdorf
In Matthias Fladung's lab connections are examined between the genome structure of forest trees and the conversion of the information stored in the genomes as a function of environmental factors. In particular economically important traits and traits relevant to adaptation are analyzed in view of geographic variation patterns. Besides, molecular

markers are developed for evaluation of forest genetic resources as well as to the practical application for marker-supported selections and forensic applications.

I hope to profit from Matthias Fladung's long and intensive experience in monitoring neutral and adaptive diversity in trees especially from his knowledge on the analysis of geographic variation patterns in traits relevant to adaptation. On the other hand the Johann–Heinrich von Thünen Institute is one of the leading and largest institutes of forest genetics in Germany. As there are plans to increase studies in adaptational genomics among forest trees in the institue I hope that working with Matthias Fladung and collegues can open up the potential for future cooperations and that I can contribute with knowledge gained in the collaboration with Giovanni Vendramin and David Neale.

Bei Postdoktoranden

Stellungnahme zur Relevanz des Projekts für die weitere Karriereplanung („motivation letter“) *– Bitte als Anlage beifügen!*

References

Adams (2004): Junipers of the World: The genus Juniperus. 275p.
Aitken S.N., Yeaman, S., Holliday, J.A. et al. (2008) Evolutionary Applications 1: 95-101
Davis, M.B. & Shaw, R.G. (2001): Science 292: 673-679
Davis, M.B., Shaw, R.G., Etterson, J.R. (2005): Ecology 86:1704-1714
Eveno, E., Collada, C., Guevara, M.A. *et al.* (2008): Molecular Biology and Evolution 25: 417-437
Gonzalez-Martinez, S.C., Ersoz, E., Brown, G.R. *et al.* (2006): Genetics 172: 1915-1926
Hampe, A. & Petit, R.J. (2005): Ecology Letters 8: 461-467
Hamrick, J.L. (2004): Forest Ecology and Management 197: 323-335
Heuertz, M., de Paoli, E., Källman, T. et al. (2006):Genetics 174: 2095-2105
Jump, A., Penuelas, J., Rico, L. et al. (2005): Global Change Biology 14: 637-643
Kim, S.C., Rieseberg, L.H. (1999): Genetics 153: 965-977
Kramer, K., Butteveld, J., Forstreuter, M. et al. (2008): Ecological Modelling 216: 333-353
Miehe, G., Miehe, S., Will, M. *et al.* (2008): Plant Ecology 194: 157-177
Neale, D. & Savolainen, O. (2004): Trends in Plant Science 9: 325-330
Opgenoorth, L., Mao, K., Vendramin, G. *et al.* (subm.)
Petit, R.J. & Hampe, A. (2006): Annual Review of Ecology, Evolution and Systematics 37: 187-214
Petit, R.J., Hu, F.S., Dick, C.W. (2008): Science 320: 1450-1452
Rehfeldt, G.E., Tchebakova, N.M., Parfenova, Y.I. *et al*. (2002): Global Change Biology 8: 912-923
Sork, V.L., Nason, J., Campbell, D.R., *et al*. (1999): Trends in Ecology and Evolution 14: 219-224

Kostenplan mit Begründung der einzelnen Positionen	EUR
Personalmittel (Einstufung sowie tatsächlich erforderlicher Betrag, inkl. Nebenkosten. Hier sollten Mittel für halbe bzw. volle Stellen kalkuliert werden)	
1 PostDoc Bat2a (DFG Pauschale für 3 Jahre)	180.000
Laufende Sachmittel	
1. Consumables	4.000
2. Pyrosequencing 1 Plate (three species)	18.000
3. HT Resequencing/Genotyping	35.000
4. Reisekosten	23.000
Einmalige Sachmittel	
5. Toughbook	3.000
6. GPS	500
7. Expeditionszelt	500
Gesamtbetrag	**264.000**

Begründung der Kostenpositionen

1. Consumables refer to lab consumables for extraction, purification, handling of samples, and to consumables in marking the ecological plots.
2. Pyrosequencing refers to current quotes from 3rd party companies
3. HT Resequecing/Genotyping greatly depends on the ratio of the two methods we will be able to achieve. This is an average calculation taken from other Evoltree Projects.
4. Reisekosten
China – field trip: 14.000 € This includes flights from Frankfurt – Bangkok – Chengdu – Lhasa and back with overweight for samples, Renting a landcruiser/driver/guide, inner chinese Permits, Visa, Hotel costs.
Lab Vendramin: 8 trips stretched over 16 months - 500 € per trip = 4.000 €
Lab Neale: 3 months trip 5.000 €
5. The tough book is the only way to use a lab-top under field conditions in Tibet. This increases the speed of work enormously. Includes additional battery pack.
6. & 7. GPS and expedition tent are necessary for the field trip.

Erklärung

ich versichere, dass ich meine Dissertation

“Between Rear and Leading edge - Juniper woodlands on the southern Tibetan Plateau - a high mountain forest-line ecosystem under environmental change”

selbstständig, ohne unerlaubte Hilfe angefertigt und mich dabei keiner anderen als der von mir ausdrücklich bezeichneten Quellen und Hilfen bedient habe.
Die Dissertation wurde in der jetzigen oder einer ähnlichen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

(Ort/Datum) (Unterschrift mit Vor- und Zuname)

Curriculum Vitae

Persönliche Daten

Name: Opgenoorth
Vorname: Lars
Geburtsdatum: 24.07.1974
Geburtsort: Düsseldorf
Staatsangehörigkeit: deutsch

Schulbildung

1981 – 1985	Gemeinschaftsgrundschule Unterfeldhaus
1985 – 1991	Dietrich-Bonhoeffer-Gymnasium Hilden, Abschluss: Mittlere Reife
1991 – 1992	Medina Senior High School Abschluss: Diploma
1992 – 1994	Dietrich-Bonhoeffer-Gymnasium Hilden, Abschluss: Allgemeine Hochschulreife

Studium

1996 - 2003	Studium der Geographie an der Philipps-Universität Maburg
2003	Diplomarbeit mit dem Thema: “Dendroökologische Unter suchungen an Birken-Weiden Wäldern des Gobi-Altais, Mongolei.”

Promotion

2004 – 2009	Arbeiten zur Promotion bei Prof. Dr. Birgit Ziegenhagen und Prof. Dr. Georg Miehe, Philipps-Universität Marburg.