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Lack of sexual reproduction within mountain steppe populations of the clonal shrub *Juniperus sabina* L. in semi-arid southern Mongolia

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Abstract

The present study describes the reproductive ecology of the prostrate shrub *Juniperus sabina* in dry mountain steppes of southern Mongolia where stands are located at the drought-limit of the species' distributional range. Even though cones are produced in large numbers, the larger part of those collected for the study had incompletely developed embryos, and only 2.5% were viable. In germination experiments only 3 out of 2100 intact seeds germinated, suggesting that germination would be unlikely under field conditions. Correspondingly, nearly no seedlings or saplings were found in the field.

Ample evidence was found for clonal growth. Patches of similar sexes of *J. sabina* were spatially associated in the field. RAPD-fingerprinting demonstrated that patches were constituted by a single genet. As mean current growth rates were between 1.8 and 6.8 cm year⁻¹, we estimated that the largest patches found in the study area had minimum ages of 770–2940 years. Thus, establishment of seedlings may have taken place in periods defined by more favourable climatic conditions, whereas dry phases have apparently been survived by clonal growth. This combination of rare sexual reproduction with extended periods of exclusively vegetative reproduction could be a widespread strategy in the harsh conditions of the central Asian drylands.

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Keywords: Clonal growth; Juniper; Mongolia; Mountain steppes; Persistence; Reproduction

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1. Introduction

The genus *Juniperus* is widely distributed throughout dry regions of the northern hemisphere. Among conifers, junipers are most well adapted to arid conditions as their xylem is characterized by a small pore volume (Sperry and Tyree, 1990). They replace other genera towards the drought-limit of trees, and often constitute the only available source of timber and fuel wood in dry areas. Thus, junipers are exceedingly important for human subsistence in semi-arid regions and stands are widely exploited, notably in Central Asia (Farjon et al., 2001). However, rates of sexual reproduction are often found to be low as many species are known to have poor germination rates, a high fraction of parasitized cones and low percentages of viable seeds (Knopf, 1990; Garcia et al., 2000a; Yilmaz, 2001). For *J. communis* there is a tendency for seed quality to decrease towards drier or colder regions (Houle and Babeux, 1994; Garcia et al., 2000b). In contrast, rooting ability of shoot cuttings increases towards the distributional limit, with clonal growth becoming increasingly more important in juniper species growing under adverse conditions (Ward, 1982; Houle and Babeux, 1994; Houle and Duchesne, 1999).

Asexual reproduction is assumed to be favoured in marginal environments (Peck et al., 1998). In plants, clonal growth and associated longevity are particularly common under harsh conditions or in otherwise peripheral populations (Eriksson, 1996; Garcia and Zamora, 2003) where sexual reproduction is severely hampered. In these cases, clonal growth still allows persistence of populations and ensures at least mid-term survival (Honnay and Bossyut, 2005). Clonally growing populations have been described for extreme environments in both high-altitude regions (Bauert et al., 1998; Escaravage et al., 1998) and central Asian semi-deserts (Song et al., 2002).

For the present study we assessed the relative importance of sexual reproduction vs. clonal growth for *Juniperus sabina* L. growing under the strongly semi-arid and continental conditions of Mongolia. In the south-eastern Gobi Altay, *J. sabina* forms extensive patches on open slopes and is the only common woody plant. One focus of our research was to analyse seed production and germination behaviour, since an initial study suggested that recruitment of *J. sabina* seedlings is virtually absent in the field (Wesche et al., 2005). We additionally analysed the genetic structure of juniper patches by means of RAPD-fingerprinting. We hypothesized that genetic diversity within patches would be low if patch extension and reproduction occurred mainly by clonal growth. On the other hand, juniper seedlings are often found beneath adult plants; be it because of the provision of safe sites (Callaway, 1997; Chambers, 2001), spatially limited dispersal or of seed hoarding by rodents (Chambers et al., 1999; Houle and Duchesne, 1999; Garcia, 2001). Patches would therefore be expected to comprise several genets if any of these aspects were to be of major importance for *J. sabina*.

1.1. Study species and study region

J. sabina's (Cupressaceae) distribution range stretches from the Mediterranean region to eastern Siberia (Meusel et al., 1965). The subspecies *J. sabina arenaria* in

Mongolia is probably the most drought-tolerant of all Asian juniper species (Farjon et al., 2001). Young plants are characterized by having needle-like leaves which develop into scale-like leaves after 10 years (Hegi, 1965). They have a prostrate and creeping growth form, which develops into a centrifugal pattern forming elliptical patches of up to 100 m in diameter. In contrast to Hegi (1965), who describes the species as being both monoecious and dioecious, plants were found to be exclusively dioecious in our study region. The small hanging cones are green in the first year, turning to dark blue in the second. Ripe cones are roughly 4–6 mm in diameter and contain 2–6 seeds, which are mainly dispersed by birds. Since the stem apices are very poisonous due to their content of sabinol ($C_{10}H_{15}OH$), diacetyl ($CH_3COCOCH_3$) and sabinen ($C_{10}H_{16}$), they are not browsed by livestock. Junipers have a sacred status in Buddhist mythology, so Mongolians tend only to collect shoots for ceremonial burning. As the impact of this practice is limited, *J. sabina* is currently not directly threatened by land use practices in the Gobi Altay.

The study area encompasses the Gobi Gurvan Sayhan National Park (GGS NP) located in southern Mongolia. At some 27 000 km² it is the second largest protected area in Mongolia (Bedunah and Schmidt, 2000) and covers deserts, desert steppes and mountain steppes. The study was carried out in the two easternmost ranges of the Gobi Altay, which are surrounded by desert steppes. In this region *J. sabina* is restricted to steep south-facing slopes at altitudes of 2300–2700 m a.s.l. (Wesche and Ronnenberg, 2004; Wesche et al., 2005). Soils are generally shallow as scree movement in freeze and thaw cycles is the dominant erosive process. The climate in the GGS NP is semi-arid and highly continental with a pronounced seasonality. The nearest governmental weather station, Bayandalay (1570 m a.s.l.), reports a mean annual temperature of 4.5 °C (mean temperature in July 20 °C, in January –18 °C; National Meteorological Service of Mongolia). Frosts occur regularly from September to May. Mean annual precipitation at Bayandalay is 110 mm, but higher in the mountains; short-term measurements by Retzer (2004) suggest that annual means might total 130 mm at 2300 m a.s.l. Precipitation shows a high inter-annual variability (Stumpp et al., 2005). Although 2001 was a drought year, the present study was performed under the more typical weather conditions of 2002.

2. Methods

2.1. Sexual reproduction

We chose two study areas of some 100 ha each in the two south-easternmost ranges of the Gobi Altay (Dund Sayhan; 43°37'28"–43°36'5"N, 103°46'1"–103°47'2"E, Zuun Sayhan; 43°28'46"–43°29'19"N, 104°4'30"–104°5'14"E). Sample plots were selected using randomly generated UTM-coordinates and a GPS. Site characteristics, including exposure, were measured for each site as part of a general study on community composition (details in Wesche and Ronnenberg, 2004). In cases where *J. sabina* was present at a particular spot we recorded sex, size and shape of patch, as well as presence of cones and seedlings for the focus patch and its four

nearest neighbours. For shoots with needle-like leaves, we double-checked the connection to the adjacent patch by excavating it. Plants develop by centrifugal growth, so patches were treated as being separate if shoots radiated from two clearly different origins, and patches had clear gaps between them. As cones are always produced in huge numbers, we counted them for two patches distinguishing between green and blue cones. These were further differentiated into intact cones and cones infested by parasites based on the presence of bore holes. The ripe blue ones were additionally divided into fleshy and dry cones. Both sampled patches had southern exposures, the first one on a moderately inclined slope (12°) and the second one in a small valley (3°). In 2004 we reassessed the presence of seedlings.

We tested viability of blue, but dried, seeds (3 × 50 seeds) with a tetrazolium test (Baskin and Baskin, 2001), in the course of which we cut open the seed and examined the embryo and nucellus status; none were found to be viable. Thus, for germination tests only ripe and fleshy cones were collected from 10 randomly selected plants in August 2002 and 2004, and were stored for 1 month in cotton bags. Germination tests were performed in the laboratory using morphologically intact cones and seeds. In 2002, dormancy was tested using both intact cones and bare seeds, both of which were put on filter paper in Petri-dishes and incubated in climatic chambers at temperatures of 8 °C/4 °C and 20 °C/10 °C (12 h of light/12 h of darkness; $n = 2 \times 50$ cones or seeds). As juniper seeds are known for post-dispersal ripening and strong dormancy (Chambers et al., 1999; Garcia, 2001), cones were kept in either dry cotton bags or moist sand at different temperatures for various intervals (Table 1). Additionally, we tried to break dormancy by submerging cones and seeds for 90 min in sulphuric acid, in liquid nitrogen (−96 °C, 5 min), or in hot water (10 min). Fifteen batches of 50 seeds each were mechanically scarified by cutting a hole (1–2 mm) into the seed coat. All germination tests on these treatments were carried out at temperatures of 20 °C/10 °C (12 h of light/12 h of darkness; $n = 5 \times 50$ seeds). The

Table 1
Treatments for *Juniperus sabina* seeds and mean fraction of seeds still viable after treatment ($n = 5 \times 50$)

Storage		Treatment	Viability (%)	Germination (abs.)
Cotton bag, dry	6 months 18 °C	Control	3.7	0
		H ₂ SO ₄ (95–97%)	2.0	0
		Water 100 °C	4.0	0
		Liquid N ₂	4.0	0
		Scarified (cut)	2.0	2
Moist sand	6 months 18 °C	Control	6.0	0
		Scarified (cut)	0.7	0
	30 months 18 °C	Control	5.2	Not tested
		Control	7.3	0
	3 months 18 °C/3 months −18 °C	Control	7.3	0
		Scarified (cut)	2.0	1
No storage		Dry cones	0.0	Not tested
Collected in 2004	No storage	Intact cones	4.4	Not tested

experiments were stopped after 120 days and viability of ungerminated seeds was assessed using the tetrazolium test. The test was repeated after $2\frac{1}{2}$ years of dry storage and data was additionally compared with viability data from ripe seeds collected in the area in 2004. This was a moist year that had followed 2 years of average precipitation levels.

2.2. Vegetative reproduction

We confirmed the known capability to form adventitious roots (Gumbatov, 2001) by putting three cuttings of *J. sabina* in water from a nearby well and exposing them to ambient temperature and light for 1 week. Growth rate under field conditions was assessed using two methods. Firstly, we randomly selected six shoots and excavated as much of them as possible. Age of shoots and calculation of average growth rate was based on counting tree rings near the shoots' base, since rings are quite observable thanks to the seasonal climate of Mongolia (Jacoby et al., 2000). Secondly, we tagged 108 shoots from nine randomly selected patches on the 27th of May and measured length increment 75 days later.

In order to check whether patches are formed by one or several individuals, one *Juniperus* patch was fully excavated. Since shoots were found to be interconnected, clonal growth was subsequently assessed with RAPD-fingerprinting. This method has been successfully employed for taxonomic studies on Asian junipers before (Adams and Demeke, 1993; Adams, 2000; Hsiang and Huang, 2000), and was also previously used to map genets within clonal species (Steinger et al., 1996; Herben et al., 2001). As our main objective was to assess genetic diversity within patches, we randomly collected 10 leaf samples from each of six widely spaced (> 100 m) juniper patches. Genomic DNA was extracted from 25 mg portions of silica-dried leaf material following the protocol described by QIAGEN (2000; DNeasy Plant Mini Kit, p. 15). Amplifications of DNA were performed in reaction volumes of 10 μ l containing 0.8 μ l DNA (10 ng μ l⁻¹), 0.6 μ l of primer (ROTH), 1 μ l of each dNTP (PEQLAB), 1 μ l 10 \times buffer (QBIOSGENE), 0.1 μ l Taq Polymerase (5 U μ l⁻¹, QBIOSGENE) and 6.5 μ l H₂O. PCR was carried out in an Eppendorf Mastercycler Gradient, which was programmed for one cycle of 2 min at 94 °C followed by 40 cycles of 12 s at 94 °C, 45 s at 36 °C and 120 s at 72 °C with a final cycle of 7 min at 72 °C. DNA fragments were separated by electrophoresis in 2% agarose gels with a Tris–acetate–EDTA (TAE) buffer system at 150 V for 150 min and stained with ethidium bromide. DNA bands were then visualized by UV light and documented using a video camera. In order to ensure reproducibility, each sample was run in at least two independent RAPD-PCR amplification reactions. Gel pictures were analysed with the software Cross Checker Version 2.91 (Buntjer, 1999); only bands in the range between 200 and 2000 bp were scored. An initial screening of 100 RAPD primers (ROTH sets A, B, D, J, N) was performed in order to test amplification profiles for polymorphism, readability and reproducibility. This resulted in the selection of four primers (A18: AGGT-GACCGT; D05: TGAGCGGACA, J14: CACCCGGATG, J15: TGTAGCAGGG) for use in the analysis.

2.3. Statistical analysis

Univariate comparisons between patches found in the field were based on non-parametric statistics, i.e. χ^2 -tests for qualitative data and Kolmogorov–Smirnov–Z-tests for quantitative data; in which case significances were based on a Monte Carlo procedure (10 000 permutations). Exposure was partitioned into “northness”, i.e. the cosine of the aspect in degrees, and “eastness”, i.e. the sine of the aspect in degrees. RAPD data was converted into a matrix which included presence(1)/absence(0) data for all the scorable bands. Statistical analyses were based on Nei and Li (1979) distances among RAPD phenotypes; clustering was done with the UPGMA method (Unweighted Pair Groups Method, Jongman et al., 1995). Statistical analyses were performed using SPSS 10.0 (SPSSinc., 1999) and MVSP 3.1 (Kovach, 1995).

3. Results

3.1. Spatial structure and sex distribution

Out of a total of 145 randomly chosen coordinates, 59 fell on a *J. sabina* patch. Individual patches were always populated by a single sex; both sexes were equally represented between patches (32 male, 27 female, χ^2 -test, $p > 0.5$). Size of focus patches ranged widely (0.1–795 m², median 13.9 m²), but male and female patches did not differ in size (Fig. 1a, Z-test, $p > 0.3$). Sexes were spatially associated in the field. Female focus plants had a significantly higher number of female neighbours (Fig. 1b, Z-test, $p < 0.001$), and male focus plants also had more similar-sexed neighbours (Fig. 1c, Z-test, $p < 0.001$). The total cover of similar-sexed neighbours was also higher for focus plants of both sexes (Fig. 1d and e, Z-test, $p < 0.001$). When only the nearest neighbours were analysed, proximity was higher for pairs of the same sex in male focus plants (1.0 m vs. 2.5 m on average), whereas this difference was negligible for female focus plants (1.3 vs. 1.4 m).

Both sexes avoided northern exposures (Fig. 2). Northness of sites, i.e. the cosine of the aspect in degrees, was significantly lower for female than for male plants (median -0.78 vs. median -0.29 , Z-test, $p < 0.001$), but exposures did not differ with respect to the west-easterly direction (sine of the aspect in degrees, Z-test, $p > 0.3$). Both sexes preferred moderately steep slopes (median inclination 13° for males, 11.5° for females) without any apparent preference between sexes (Z-test, $p > 0.8$).

3.2. Seed production and germination

All male individuals flowered; all female individuals produced abundant cones. The majority of cones were blue on the patch studied in more detail on the steep slope (Table 2). However, the majority of cones were found to be dry (64.7%), while a total of 8.6% were infested by parasites. At the valley site most cones were green, 37.5% of all cones were infested by parasites, and 31.8% were dry.

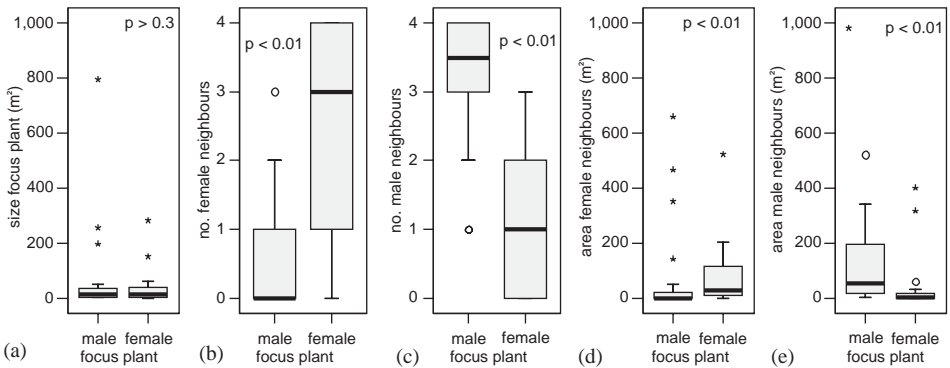


Fig. 1. Differences in size and numbers among sexes of *J. sabina* plants. (a) Size of focus plants (a male outlier of 795 m² is not shown to avoid compression of y-axis); (b) mean no. of female neighbours; (c) mean no. of male neighbours; (d) mean size of female neighbours; (e) mean size of male neighbours (Box and Whisker plots with median and inter-quartile range, stars indicate outliers, in (e) two male outliers of 1250 m² and 1939 m² were not shown to avoid too strong a vertical compression of boxes).

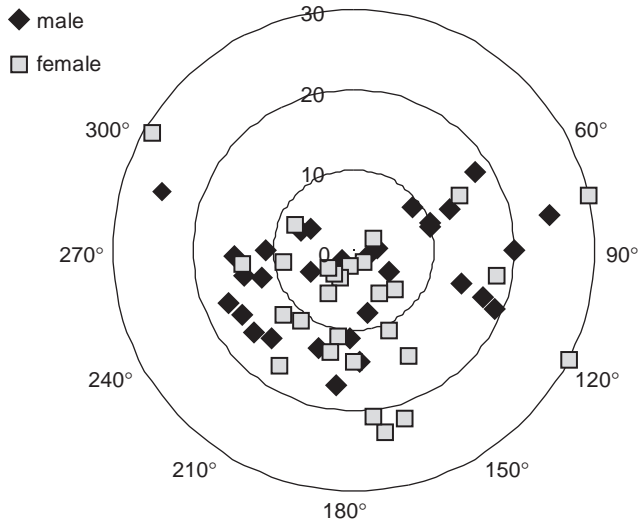


Fig. 2. Distribution of sexes among 59 patches of *J. sabina* (32 male, 27 female) with respect to aspect and slope inclination (degrees, indicated by concentric circles).

Although all candidates were excavated, neither saplings nor seedlings were found among the 59 patches analysed in 2002 (plus an additional 213 neighbours). However, a single sapling was found in 2004. The plant was growing in the bed of a temporary brook, and was clearly separated from *J. sabina*'s principal habitat on scree slopes.

In the germination experiments conducted on fleshy seeds, only three out of the 2100 tested seeds germinated; one on the 60th and two on the 90th day. All three had

Table 2

Ratio of unripe (green), ripe (blue) and damaged cones for two *J. sabina* individuals situated on a scree slope and in a valley bottom

Cones	Steep slope		Valley bottom	
	Number	%	Number	%
Green, intact	484	17.1	2584	14.1
Green, infested by parasites	67	2.4	1363	7.4
Blue, intact	276	9.7	3050	16.6
Blue, infested by parasites	175	6.2	5532	30.1
Blue, dry	1835	64.7	5830	31.8
Sum	2837		18359	
Size of the patch in m ²	5.4		14.3	
Cones/m ²	525.4		1283.9	

been mechanically scarified (Table 1); none of the other treatments appeared to be effective in breaking dormancy. TTC-tests of the ungerminated seeds showed that, on average, only 2.5% of the seeds were viable. Dissection demonstrated that all stages of embryo and nucellus decomposition were present. Moist and cold storage resulted in a slightly higher seed viability than dry storage, whereas scarification reduced viability to between 0.7% and 2%. The overall low viability was also confirmed for seeds collected in 2004. According to TTC-tests, viability of fleshy ripe cones was 4.4%. Germination treatments had no significant impact on viability (Kruskal–Wallis test, $p = 0.144$) and viability was also not increased after $2\frac{1}{2}$ years of storage in sand (Z-test, $p > 0.8$). Seeds collected in 2004 did not have a higher viability than those collected in 2002 (Z-test, $p > 0.9$; Table 1).

3.3. Clonal growth

The excavated juniper patch was one, albeit rather small (size 9.8 m²), clone. All roots and shoots were still connected to one origin which was located on the upper border of the patch with all shoots growing downwards from there. The basal shoot was broken on its base and we could not find the connecting part. Shoots are capable of re-rooting after fragmentation since all cuttings taken in the study were found to develop adventitious roots after less than a week in water.

RAPD analysis yielded 27 scorable bands. Cluster analysis of the genetic pattern revealed six distinct groups (Fig. 3), however, overall similarity among patches was relatively high (maximum distance among groups 0.20). Samples from patches 1–4 and 6 belonged to the same RAPD-phenotype. The small difference between the two samples from patch 5 reflects presence/absence of just one band, thus minimum similarity within this patch was 97% rather than 100%.

The longest branches were found to be 22–84 years old, with counts of tree rings at the given shoot's base and shoot length indicating annual shoot elongation rates of

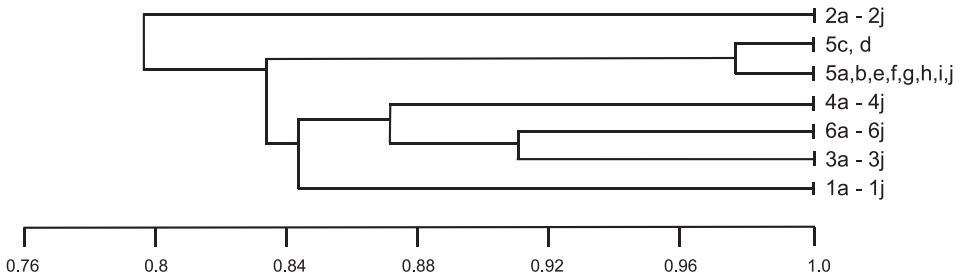


Fig. 3. UPGMA-cluster diagram (coefficient: Nei and Li, 1979) of the RAPD data of six *J. sabina* patches. Labels on the right side of the diagram indicate patch membership.

1.7–6.8 cm (mean 4.42, S.D. 1.57). Mean shoot elongation directly measured in the months May–August 2003 was found to be 1.48 cm (S.D. 0.8).

4. Discussion

4.1. Sexual reproduction

Complete absence of young growth can hardly be stated with certainty in a study region of this size, but several indicators suggest that under the present climatic conditions sexual reproduction of *J. sabina* is exceedingly rare. The almost complete lack of seedlings in the field corresponds well to low levels of seed viability and poor germination. Cone numbers are huge, but less than a third of the cones are morphologically intact with parasitism being a major factor (Table 2). High infection rates are reported for a number of Eurasian junipers (Farjon et al., 2001; Garcia et al., 2000b), and species of the genus *Megastigmus* (Hymenoptera, Torymidae) are the most commonly reported parasites from Europe to Central Asia (Vikberg, 1966; Roques, 1983; Turgeon et al., 1994; Sultanov, 2001).

Among the morphologically intact seeds a high proportion revealed successive stages of embryonic decomposition, and only 2.5% were viable; a phenomenon also found in other junipers. In a study in Turkey, 98% of *J. foetidissima* seeds had aborted embryos (Knopf, 1990), but quality of cones was dependent on site conditions as the ratio of viable seeds was clearly higher on fertile soils. Studies on the reproductive biology of *J. oxycedrus* revealed that low seed viability is caused by low pollen vigour or pollen viability (Arista et al., 2001). The pollen triggers the formation of the seed but dies before fusing with the ovary, resulting in the abortion of the seed. Whether this is the principal mechanism explaining the number of dead seeds in junipers remains to be studied.

Dryness of climatic conditions is one of the most important constraints for tree formation (Pigott and Pigott, 1993) and has been shown to influence reproductive behaviour in peripheral populations of junipers (Garcia et al., 2000b). On a continental scale, stands in the Gobi Gurvan Sayhan clearly form outposts at

J. sabina's drought limit. Theoretically, the severe drought of 2001 may have caused an unusually inferior crop in 2002, as cones of *J. sabina* need 2 years for maturation, which would have subsequently resulted in an underestimation of the overall importance of sexual regeneration as calculated from our figures for 2002. Given that neither seedlings nor saplings were common in the region, we doubt that germination and establishment have occurred in other years. This is also supported by the equally low viability of seeds collected in 2004, which ripened under relatively moist conditions.

Thus, we couldn't find an impact of annually varying precipitation on seed viability. In contrast, Garcia et al. (2002) described varying seed sets among years related to different precipitation levels for Spanish populations of *J. communis*. However, these local changes in seed output were small compared to changes recorded for large-scale geographical differences (Garcia et al., 2000b), suggesting that overall seed quality is mainly controlled by the regional macroclimate rather than by local inter-annual changes. Correspondingly, populations of *J. sabina* growing at 600 mm annual precipitation (compared to the 130 mm in our study region) had a comparatively high seed viability of 50% (Verdú et al., 2004).

Other abiotic factors might also influence cone maturation, though it is not clear which stage of seed development is particularly sensitive. Houle and Babeux (1994) studied seed viability of *J. communis* along a north–south gradient in Canada and report that a short vegetation period enhances the abortion rate of seeds. Temperature and length of growing period were described to constrain sexual regeneration in other parts of the northern hemisphere (Chambers et al., 1999; Garcia and Zamora, 2003). Age is also known to detrimentally affect seed quality in junipers (Ward, 1982). As demonstrated by Roloff et al. (1990), germination rate was 30–70% for seeds from younger trees, but only 5% for older trees of *J. communis*. Importance of aging has, as yet, not been tested for central Asian junipers. Judging by our growth measurements, plants of *J. sabina* are possibly several hundred years old (see below); thus there may indeed be an effect of aging on seed quality.

The low number of viable seeds dramatically reduces the potential number of diaspores capable of germination. Of the 50 seeds found to be viable in 2002 only three actually germinated. Several types of dormancy are possible (cf. Baskin and Baskin, 2001), but due to the overall low numbers of germinated and viable seeds in our study, no significant differences could be detected for any treatment. The fact that only scarified seeds germinated indicates a mechanical dormancy that might be broken in situ by the cracking of seeds during freezing and thawing cycles on the mainly southerly exposed habitats, or by the impact of moving scree on the slopes as shown e.g. by Ibanez and Passera (1997) for *Anthyllis cytisoides* in Spain. Moreover, junipers, including *J. sabina*, are mainly dispersed by birds (Jordano, 1993; Chambers et al., 1999; Garcia, 2001), and gut passage also leads to modifications of the seed coat and enhanced likelihood of germination (Traveset and Verdú, 2002). Thus, scarification of the seed coat probably occurs under natural conditions.

Storage had no effects on seed viability of *J. sabina*. This is in contrast to findings by Chambers (2001), who describes that embryos of *Juniperus* species often complete their development much later than maturation of the cone. Even fully matured seeds

would still need constantly moist sites for germination. Since we had no seedlings, we did not assess establishment in *J. sabina*, but it is likely that drought also affects seedling development rendering successful reseeded an extremely improbable event under the prevailing conditions in southern Mongolia. Unlike other junipers (Verdú et al., 2004), even mother plants do not seem to facilitate reseeded in *J. sabina*, as results from the genetic analysis indicated a genetically identical structure within patches.

4.2. Clonal growth

Our results suggest that non-sexual growth is the main mode of survival and reproduction of *J. sabina* in the Mongolian Gobi desert. The cluster analysis clearly demonstrates that 5 out of 6 visually separated patches were formed by a single RAPD-phenotype, and thus, most likely by one genet. The exception was patch 5, where minimum similarity was slightly lower due to the difference of only one band. We do not know whether this variation is the result of either a somatic mutation likely to occur in long-living organisms (Loxdale and Lushai, 2003), or of the method itself, as DNA mismatch as well as small pollutants might lead to a different RAPD profile and overestimation of genet diversity. However, we can assume that at least five, if not all patches sampled, were formed by one single genet. The very fact that the overwhelming number of shoots sampled showed identical fingerprints within a patch suggests that RAPD-fingerprinting is an adequate method for mapping genets (Steinger et al., 1996; Herben et al., 2001), and that the widely ascertained problems with reproducibility of RAPDs are possibly exaggerated.

The results of the genetic analysis are also supported by other data gathered during the study. Clonal reproduction is possible as cuttings rapidly developed adventitious roots in water, and planting of cuttings is one of the principal approaches for propagating Asian and North American junipers (Houle and Babeux, 1994; Gumbatov, 2001; Kosmyin and Tezekbaev, 2001). In addition, *J. communis* in Europe is known to grow by layering (Roloff et al., 1990). Invasion of patches by neighbouring plants is apparently rare, as our stands were always formed by a single sex and most likely constituted one individual. Still, our data differ from a study of sexually reproducing populations of prostrate *J. communis* in Canada (Houle and Duchesne, 1999). There, clumping was also observed but was attributed to the limited dispersal of seeds. Sexes had no positive spatial relation, and repulsion among male specimens was ascribed to competition.

The association of patches with similar sexes found in the Gobi Gurvan Sayhan National Park (Fig. 1) is most easily explained by assuming that a large part of the neighbouring patches belong to one individual that had been fragmented. The small patch we excavated supported this idea since it belonged to one individual. As the basal shoot had apparently been torn, it is quite likely that it had previously been part of a larger clone and had possibly slid down the unconsolidated scree at some point. Since *J. sabina* is restricted throughout its distributional range to open slopes (Hegi, 1965) where substrate movement can be expected to be omnipresent, we

assume that fragmentation and re-rooting is likely to be a common strategy of reproduction in that species.

An interesting question arising from the results of our study is the possible date of the establishment of *J. sabina* seedlings. There is no direct approach to this question in our case since individual branches have a shorter life-span than the genet. Moreover, the origin of a patch is not easily found and a wide variety of disturbances could cause patch fragmentation. A problem with age estimates based on the size and growth rates of the genets is the fact that growth can hardly be expected to be constant over centuries. Thus, results have to be treated with care (Steinger et al., 1996). In our study, data on mean annual growth obtained from older branches and direct growth measurements of shoots between May and August 2003 gave comparable results when we include further un-recorded growth which occurred after August 2003 (the growth period in this region continues until September, Borisova and Popova, 1985). Thus, we can assume that both methods used to estimate current growth rates of *J. sabina* patches provided reliable data, at least for a period comprising the last few decades.

Maximum diameter of visually discernible patches of *J. sabina* in our study area was 100 m, and if we assume lateral growth, growth measurements suggest a minimum age of 770–2940 years for these larger patches. This is a conservative estimate since many patches appeared to grow in only one direction (mostly downwards) from a single origin. Moreover, data on size always referred to single, clearly separated patches; if neighbouring patches had belonged to the same genet, calculations would have yielded much greater ages. In any case, estimates ranging from a few decades to up to 3000 years are generally realistic. In Kyrgyzstan, mean ages of *Juniperus* species (including *J. sabina* and *J. communis* among others) are between 500 and 700 years; with maximum ages of up to 2000 years (Buttoud and Yunusova, 2001).

At such time scales, constancy of climatic conditions can no longer be assumed. For Mongolia, climatic measurements and tree-ring analyses by Jacoby et al. (2000) demonstrate a trend towards higher average temperatures in autumn, winter and spring during the last 40 years. In contrast, summer conditions have become slightly cooler. However, as recent changes have been well within the variance of the last 450 years, data does not necessarily indicate a major trend. Nevertheless, paleo-climatic studies summarized by Gunin et al. (1999) show large-scale climatic variations during the Holocene in Mongolia. According to these data, which are based on pollen-profiles and lake-level analyses, the climate between 8000 and 5000 BP, and around 1800 BP, was moister than it is today. If we assume that moisture availability is a limiting factor for sexual reproduction in *J. sabina*, then establishment of the oldest genets might well date back to 1800 BP.

5. Conclusions

Extreme conditions are expected to be one principal factor responsible for the lack of sexual reproduction of *J. sabina*. Being extremely long-lived, populations survive

adverse conditions and persist by vegetative growth. This is apparently a widespread strategy in Asian drylands as the majority of the most important grasses of Asian deserts and semi-deserts are long-lived, and several are known to grow clonally (Lavrenko and Karamysheva, 1993; Hilbig, 1995; Liang et al., 2002; Huang et al., 2004). Moreover, the relative importance of clonal herbaceous species increases towards the drier steppe types and deserts of Central Asia (Song et al., 2002). For junipers, Houle and Babeux (1994) assume a negative trade-off between the quality of seeds and the capacity to form adventitious roots in *J. communis*; and Gumbatov (2001) emphasizes a similarly inverse relationship between intensity of vegetative regeneration and the amount of cones produced for several junipers in Azerbaijan. More recently, Bruelheide (2003) described that *Populus euphratica* (*P. diversifolia*) in the Taklamakan desert propagates by extensive clonal growth, and data by Qong et al. (2002) suggest that a similar strategy is favoured by *Tamarix ramosissima* in the same landscape. Thus the reproductive behaviour of *J. sabina* represents a seemingly wide-spread strategy of plants living in the cold deserts of central Asia.

Drylands represent only one particular harsh environment with limiting factors in terms of sexual reproduction. Both theory and empirical evidence suggest that multiple regenerative strategies, with phases of sexual reproduction in favourable conditions and vegetative growth in adverse conditions, are widespread in perennial plants (Eriksson, 1996; Garcia et al., 1999). Clonal growth allows persistence of populations under high levels of abiotic stress and/or competition (Garcia and Zamora, 2003), and is also common in remnant populations which constitute outposts in the distributional range of any given species (Eriksson, 1996). Prolonged clonal growth might ensure mid-term survival but may also lead to reproductive collapse and eventually to monoclonal populations. On average, such populations would be expected to show reduced capacity to adapt to changing environmental conditions, or an otherwise reduced level of vitality (Honnay and Bossyut, 2005). Thus, clonal growth as a strategy has its dangers.

However, in short- and mid-term time-scales, clonal growth can greatly increase resilience of plant populations, and in the case of key-stone species, whole communities (Eriksson, 2000; Garcia and Zamora, 2003). In the south-eastern Gobi Altay, stands of *J. sabina* provide above-average levels of soil nutrients, which are used by a set of associated species (Wesche and Ronnenberg, 2004). Increased levels of fuel wood collection pose a theoretical danger, as recolonization by *J. sabina* is extremely unlikely. However, if current levels of human impact are maintained, lack of sexual reproduction should not be regarded as alarming, and, as it is climatically controlled, is beyond control of conservation efforts anyway.

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