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Seed production and germination of three rare *Saussurea* species in the Kuznetsk Alatau mountains (Russia)

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ABSTRACT

Background: Reproductive traits play a major role in maintenance of long-lived perennials. Many studies suppose that the rarity is concerned with the most vulnerable phases of plant life-cycle. We ask if the scarcity of rare *Saussurea* species, *S. baicalensis*, *S. salicifolia* and *S. schanginiana*, is the consequence of the way they reproduce. **Results:** Seed production and two *in vitro* germination tests of these species were investigated in the Kuznetsk Alatau mountains. High seed productivity and germination of seeds stratified by cold but dramatically low abundance of generative shoots are featured for *S. baicalensis*. *S. salicifolia* is characterized for higher seed productivity per 1 m² in comparison with two other species. However, its seed germination is very low in both tests. *S. schanginiana* differs critically low abundance of generative shoots, too few mature achenes per 1 m² and high germination of seeds freshly harvested and stratified by cold. **Conclusion:** The studied species are locally endangered within the Kuznetsk Alatau mountains, and their ability to seed reproduction is low, so they are vulnerable and need conservation.

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INTRODUCTION

The genus *Saussurea* DC. (Asteraceae) includes approximately 300 species inhabiting Eurasia and North America although natural habitats of the majority of its species are located in Asia [1]. The largest number of *Saussurea* species is confined to mountain areas and occurs on the upper bound of vegetation. There is a natural decrease of taxa's number from the Himalayas and China's mountain ranges to the Western Europe and the North America [2]. So mountains of Siberia especially the Altai-Sayan mountain system are characterized by considerable diversity of *Saussurea* species [3, 4]. Except *Frolovia frolovii* (Ledeb.) Raab-Straube separated into the new genus *Frolovia* (DC.) Lipsch. [5] thirty-three *Saussurea* species grow in the Altai-Sayan mountain system within the Russian Federation. Among them *S. ceterachifolia* Lipsch., *S. dorogostaiskii* Palib. emend Krasnob. et V. Khan. and *S. jadrinzevii* Kryl. are included in the Red Book of Russian Federation [6]. Thirteen *Saussurea* taxa have accepted as being in need conservation in Russian red books of regional levels [7]. In a Web of Science and SCOPUS searches in September of 2014 there is a lack of papers examining ecological traits of *Saussurea* species inhabiting Russia despite evidences of their rarity. Besides researches of chemical composition suggests that Siberian *Saussurea* species are very promising for medicine [8, 9, 10, 11, 12, 13]. The clinic gave positive results in the treatment of giardiasis hepatocholecystitis and enterocolitis by tincture of *S. salicifolia* (L.) DC. [14, 15]. Also it is reasonable to consider this species to be perspective in the treatment of malignant tumors due to it contains phenolic compounds and lignan arktigenin [16, 17, 18]. *S. salicifolia* common known in Siberian folk medicine as "golubushka" is widely used as an anthelmintic and giardiasis remedy and exposed to procurement of raw materials by local population. Natural resources protection is an important additional cause to investigate rare species of *Saussurea*. In view of necessity of such researches we studied morphology, seed production and *in vitro* germination of three rare *Saussurea* species known from sporadic localities. *S. baicalensis* (Adams) Rob., *S. salicifolia* and *S. schanginiana* (Wydł.) Fisch. ex Herd. were observed in the Kuznetsk Alatau mountains. The Kuznetsk Alatau is the system of low, medium and high mountain ranges in the south of Western Siberia extending for a distance of about 300 km from north to south

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and a width of 150 km from east to west. The relatively small area of this mountainous region sufficiently bears signs of the vegetation of the Altai-Sayan mountain system. Therefore, we regard it appropriate to consider the Kuznetsk Alatau as a key area for studying reproduction traits of rare *Saussurea* species.

Saussurea baicalensis, *S. salicifolia* and *S. schanginiana* have large geographical ranges covering mountainous regions of West and East Siberia, Kazakhstan, Mongolia and China [19, 20]. All three species have restricted habitat specificity. *S. baicalensis* inhabits alpine and subalpine meadows, thickets of shrubs, tundra and stony placers in high mountains. *S. salicifolia* occurs mountainous and flat steppes, rocks, rocky slopes and occasionally brackish steppe. *S. schanginiana* grows in high-mountain tundra, gravelly and stony slopes, limestone rocks and rarely in dense larch or Siberian cedar forests [3]. Under our field observations abundance of these three species estimated with a six-point Braun-Blanquet scale by eye is very small in the Kuznetsk Alatau (always <1% cover). *S. baicalensis*, *S. salicifolia* and *S. schanginiana* inhabit a few localities in the Kuznetsk Alatau mountains and occupy the small fraction of their potential suitable habitats. So according Rabinowitz's [21] scheme *S. baicalensis*, *S. salicifolia* and *S. schanginiana* are sparse species, and they are locally endangered by the extended model of commonness and rarity by Rey Benayas *et al* [22].

Studying of plant life-cycles allows to suggest a hypothesis where is the weakest link causing the rarity. The best strategy for mountainous especially alpine habitats is to occupy the space as long as possible after establishment in a new site. So a long-lived perennial is more preferable life form for alpine plants [23]. Long-lived perennials which spread flowering over several seasons (polycarpics) have advantages over perennials relying on a single reproductive event (monocarpics) [24]. Owing to this monocarpic perennials have few representatives in floras of high-latitude or temperate mountain ranges [25]. As for polycarpic plants, the ability to reproduce both sexually and vegetatively is of great importance in fast dissemination and stabilizing in new habitats [26]. To define monocarpy or polycarpy, and distinguish clonal growth forms from non-clonal plant morphology must be a subject of much study. Many studies focused reproductive traits and provided the best evidence of factors that correlate with rarity [27; 28; 29]. Hart [30] assumed relationships between seed production and life-cycles. The number of inflorescences per generative shoot and the number of seeds per inflorescence are modes to regulate seed productivity of the genetic individual [31]. It is controversial whether more readily quantifiable reproductive characters such as seed productivity and germination play a major role in maintenance of long-lived plants. Life-history attributes such as mean longevity, time of first reproduction, length of reproductive period, juvenile and seedling survivorship make relatively more contribution to population dynamics in the short term [32, 33]. At the same time using a comprehensive life-cycle analysis Byers and Meagher [34] established that seed production and colonization of new sites would be important in the long term survival of a rare perennial plant. The seed germination and seedlings establishment are the most vulnerable phases in plant life-cycles [35], so information about them should allow to understand the causes of the scarcity of endemic and threatened plant species and design efficient conservation strategies by analyzing data on seed germination and dormancy [36; 37]. To maximize the probability for survival seed must germinate in the exact right time of the year. So dormancy is of great importance in terms of plant fitness [38]. The commonest dormancy type for perennials is morphophysiological dormancy [39]. Seeds with this type of dormancy need first a dormancy-breaking treatment (warm, cold or both), and then a growth period (warm or cold) to germinate [40]. We ask if the scarcity of *S. baicalensis*, *S. salicifolia* and *S. schanginiana* is the consequence of the way they reproduce. For the purpose of it we investigated morphology, life-cycle, seed production and germination of these species in the Kuznetsk Alatau mountains.

MATERIALS AND METHODS

Using herbarium specimens collected earlier (TK, NS and NSK), and our own field observations, we recorded all localities of *Saussurea baicalensis*, *S. salicifolia* and *S. schanginiana* in the Kuznetsk Alatau. To examine if these species reproduce asexually by vegetative propagation we dig out 5–15 individuals of different life-cycle stages. We determined the age of *S. baicalensis*'s generative individuals with procedure of tap root slicing and growth rings counting. To estimate seed production characteristics we used the potential seed production (PSP), actual seed production (ASP) and seed: ovule ratio showing the ratio of the ASP and the PSP. The PSP is the number of ovules produced by any unit of account: an inflorescence, shoot and individual [41]. Estimation of the PSP makes it possible to characterize the reproductive capacity of the species, its ability to reproduce in populations [42]. The PSP depends on the number of generative individuals and generative shoots in a population, number of flowers in an inflorescence and ovules in a flower. The ASP, or the number of viable seeds produced by an element of the population, affects on self-sustaining of population. As a rule, it is only a small part of the PSP and depends on many abiotic and biotic factors such as pollination conditions, herbivore presence, climate change, etc., which leads to its significant variability. Due to the strong variability of the PSP and the ASP and often weak correlation between them Levina [43] offered a relative indicator named the seed: ovule ratio considered a reliable indicator of the "success" of seed breeding and adaptation of the species population. In August 2013 we counted the PSP and ASR at levels of an anthodium, shoot and individual by

recording the data from natural populations of *S. baicalensis*, *S. salicifolia* and *S. schanginiana* in the Kuznetsk Alatau (Fig. 1).

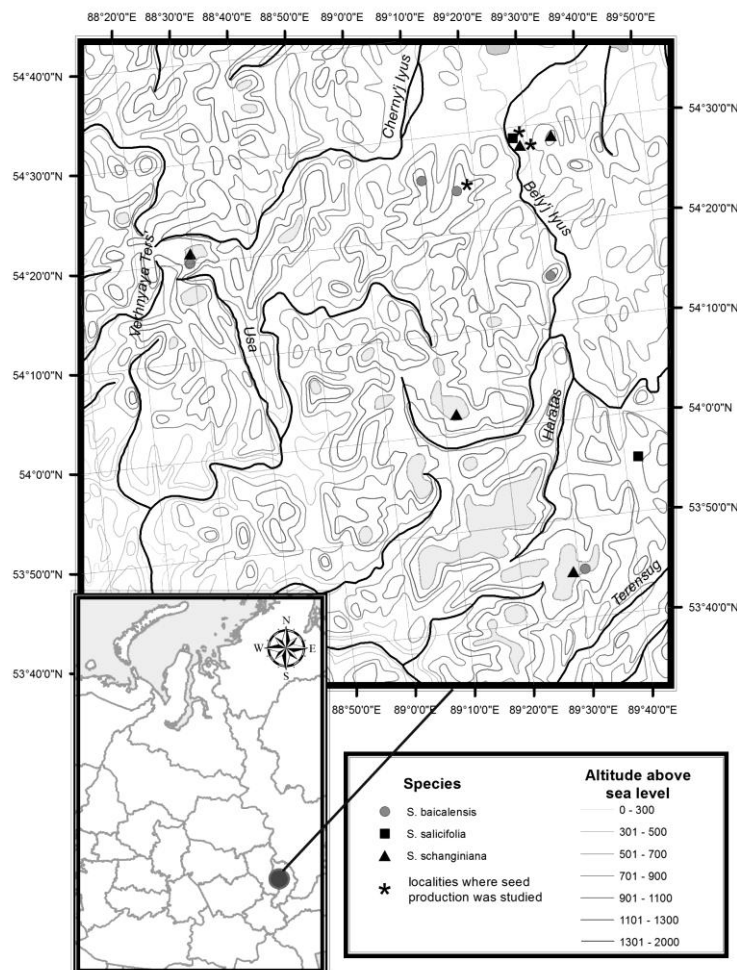


Fig. 1: Localities of rare *Saussurea* species in the Kuznetsk Alatau

In the population of each studied species we counted (1) a number of generative shoots per m², (2) number of anthodium per generative shoot and (3) number of ovules and achenes per anthodium. To evaluate parameters 1 and 2 we took measures on thirty indicators with the transect method from the centre to border of the population. Populations of *S. baicalensis* and *S. schanginiana* studied in 2013 had the critically low number of generative shoots (five in *S. baicalensis*'s and twelve in *S. schanginiana*'s ones). So we counted the number of achenes in ten *S. baicalensis*'s, two hundred and ten in *S. salicifolia*'s and twenty in *S. schanginiana*'s anthodia. Mature achenes collected from these plant species on different dates on August 2013 were subjected to germination experiments in September 2013. The part of them was subjected to stratification with cold (+2 °C) during 2 months in the attempt to break seed dormancy. We carried out standard germination test by placing seeds taken from anthodia on moist filter paper lined in 7-cm diameter petri-dish. Each trial had 3 replicates, each of 50 seeds. The test was carried out at room temperature (+22 °C) for 20 days. We placed each petri-dish on a bench near a window and watered them with distilled water regularly. Germination was recorded when the radicle emerged. In all cases we examined seeds every day for 20 days and counted all germinated seeds then removed them from the dishes. The results of a germination test were expressed in germination percentage, germinative energy (GE) and germination period (GP). GE refers to the percentage of seed in the sample that has germinated in a test up to the time when the number of seeds germinating per day reaches its peak. GP is the total period of germination. The number of days required to reach this peak is the energy period (EP). In general, seedlings that originate from seed that germinates within the energy period has the greatest chance to survive [44].

Results:

We studied the occurrence of *Saussurea baicalensis*, *S. salicifolia* and *S. schanginiana* in the Kuznetsk Alatau and charted all their localities on the map (Fig. 1). These species inhabits from two to five localities with several sparse populations in each.

S. baicalensis is a long-lived perennial monocarpic plant. It takes the development stage of a rosette plant in the beginning of life-cycle and consists of a single long shoot during flowering. The age of generative individuals varies from 5 to 7 years. Subsurface parts include long taproot and unbranching rhizome (caudex). *S. salicifolia* and *S. schanginiana* are long-lived perennial polycarpic plants. Their tops consist of rosette polycyclic vegetative and generative polycyclic monocarpic long shoots. Branching rhizome with a long taproot locates underground. All three species reproduce only sexually, vegetative propagation of *S. salicifolia* and *S. schanginiana* exhibits only as a senile fragmentation. According to our observations, generative individuals of these species are featured by intervals between flowering seasons during 1–3 years.

Among studied species the most RSP of generative shoot characterizes *S. baicalensis*. We observed also the most efficient process of achene maturation for this species (Tab. 1).

Table 1: Seed production of rare *Saussurea* species in the Kuznetsk Alatau

Seed production parameter	<i>S. baicalensis</i>	<i>S. salicifolia</i>	<i>S. schanginiana</i>
PSP of anthodium	44.40±2.28 / 29–59 (20)	16.77±0.44 / 4–34 (210)	72.3±8.9 / 36–120 (10)
ASP of anthodium	23.55±3.31 / 3–43 (20)	4.06±0.34 / 0–24 (210)	31.7±3.4 / 15–54 (10)
Seed: ovule ratio, %	53.04	47.95	43.85
Number of anthodia per shoot	9 / 5–14 (5)	3.54±0.18 / 0–17 (190)	1
PSP of generative shoot	212 / 120–360 (5)	24.93 / 0–224 (30)	31.7±3.4 / 15–54 (10)
Number of generative shoots per 1 m ²	0.005 / 0–1	2.32±0.32 / 0–12 (90)	0.062 / 0–1
Number of achenes per 1 m ²	1	87.22 / 0–2688	0.5

Note. The data are exposed in the following order: $M \pm m / \text{lim} (n)$ where M : mean; m : the standard error of the mean; lim : the fluctuation range of values of the sample, n : the sample size.

The maximum number of generative shoots per 1 m² is the feature of *S. salicifolia*. This species looks to be the most successful in the view of the number of achenes per 1 m². Critically low number of generative shoots and crop-producing power was observed in *S. baicalensis*'s and *S. schanginiana*'s populations. Small abundance of *S. baicalensis*'s individuals is associated with life-form of this species. *S. schanginiana* features low abundance due in part to the low ASP of generative shoot, kind of lengthy breaks in bloom which can last up to 3 years according to our observations.

Among studied species the highest germination of freshly harvested seeds characterizes *S. schanginiana* (Fig. 2). *S. baicalensis*'s seeds stratificated with cold exhibited the best germination. We signed the relatively low germination of *S. salicifolia*'s seeds in both versions of the experiment. Germination tests of fresh and stratificated seeds differs significantly ($p < 0,01$, Mann-Whitney U Test) only for *S. baicalensis*.

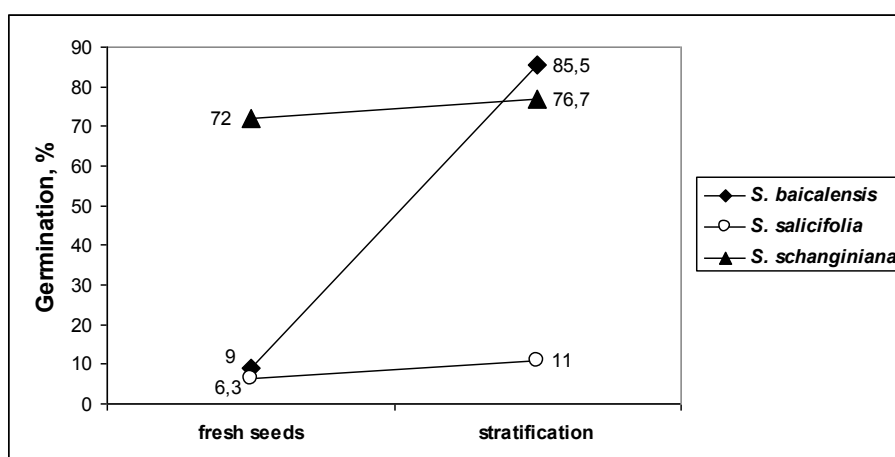


Fig. 2: Germination of *Saussurea baicalensis*, *S. salicifolia* and *S. schanginiana*

Freshly harvested seeds of *S. baicalensis* demonstrates no well-marked GE and EP the same way as *S. salicifolia*'s seeds (Fig. 3). The GE of *S. baicalensis*'s seeds after stratification accounts 62,4 % with the EP during 11 days. The GP in both cases lasts 19 days.

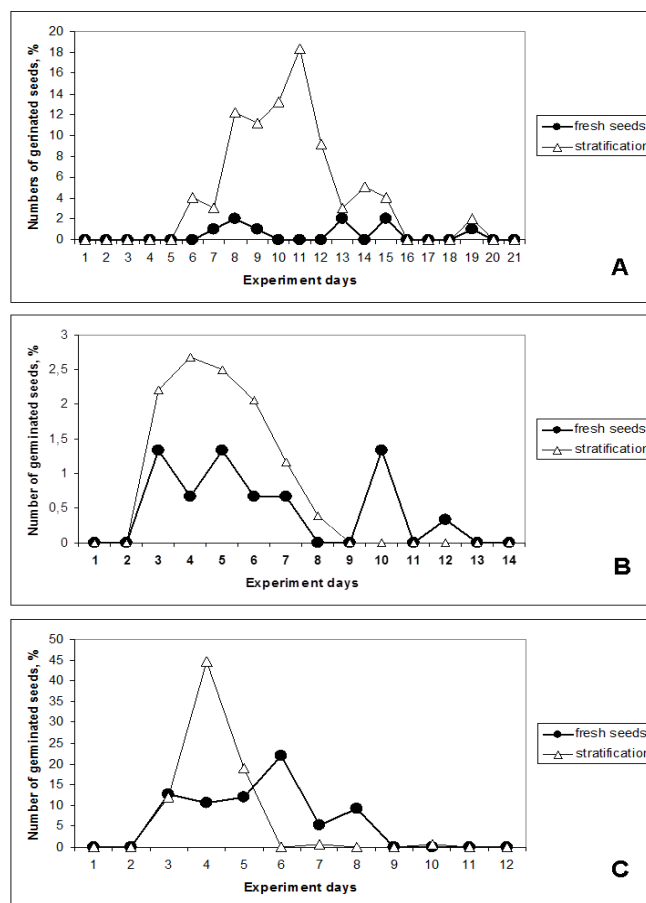


Fig. 3: Germination of fresh and stratified seeds: A – *Saussurea baicalensis*, B – *S. salicifolia*, C – *S. schanginiana*

The GE of *S. salicifolia*'s stratified seeds has low value (4,9 %) with the short EP during 4 days. The GP accounts 12 days for fresh seeds and 8 days for stratified ones. *S. schanginiana* exhibits high values of germination percentage in both cases of experiments while stratification leads to shorter EP during 4 days. But freshly harvested seeds have rather more GE totaled 57,3 % whereas stratified seeds exhibit GE of 56,6 %. However stratification reduces the GP from 8 to 5 days.

Discussion:

Saussurea baicalensis, *S. salicifolia* and *S. schanginiana* exhibit the evident rarity within the Kuznetsk Alatau and need special observations to control the state of their populations. All three species are long-lived perennial plants reproducing only sexually. But each of this three species has a weak point in the life cycle. *S. baicalensis* looks as the fittest in terms of fecundity in comparison with other studied species. It produces multiple seeds per generative shoot, and it is characterized by the well-marked morphophysiological dormancy which allows achenes to germinate at the next season after bearing. But the life form of *S. baicalensis*, a long-lived perennial monocarpic, has disadvantages such as high risks of dying before reproductive event [44]. This leads to temporal fluctuations in population demography when generative individuals have the critical low abundance (5 or less individuals per population). Hence, from time to time one can observe the very small seed productivity per area unit of *S. baicalensis* in spite of inherent for monocarpic plants fecundity and high germination. Reproduction is not fatal for *S. salicifolia* and *S. schanginiana* and these two species have the ability to make several attempts to bear seeds. *S. salicifolia* produces sufficient number of seeds per 1 m² owing to life form implying several generative shoots per individual. So its seed productivity per area unit is much more in comparison with two other studied species. However the quality of its seeds is very low. This species appears to have the weakest point in seed germination. Though we do not expect that seeds of *S. salicifolia* require a certain pre-sowing treatment more complicated than cold stratification during two months. *S.*

schanginiana features extremely low abundance of generative shoots and consequently dramatically low seed productivity per 1 m². The absence of significant seed dormancy allows us to presume that the most *S. schanginiana*'s seeds germinate immediately after bearing in the end of short mountainous summer. The probability of seedling's mortality increases as a result.

Conclusion:

The rarity of *Saussurea baicalensis*, *S. salicifolia* and *S. schanginiana* is concerned with reproductive traits which restrict population abundance and easy colonization of new sites. There are too few reasons to define whether these traits are a cause or a consequence of rarity for the time present. As whatever few localities even within large geographic range, small populations and low population abundance coupled with low seed productivity or sparse germination increase the risk of 'extinction vortex of small populations' [46]. As locally endangered species *S. baicalensis*, *S. salicifolia* and *S. schanginiana* are vulnerable and need conservation in the Kuznetsk Alatau.

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