The state of the legume reproductive system as the indicator of the species adaptive abilities

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Abstract
The study examines the legume reproduction systems of the genera Amoria, Astragalus, Chrysaspis, Glycyrrhiza, Trifolium, Melilotoides and Trigonella in the Urals, implementing various programmes of reproductive development. The study was conducted at the cellular, histological and organ levels, the implications of changing of the population reproductive strategy were assessed, as well as the structural and functional restructuring of the self-incompatibility by light and fluorescent microscopy.

Background
The species adaptive capacity is determined by the set of indicators, among which the leading role belongs to the ability to resume efficiently in a changing environment. Reproductive strategy of a species as the basis of its life strategy is a major factor in the formation and implementation of the adaptive capacity of a species. If the reproductive strategy is changed, the adaptive capacity of the population can be extended.

The need to study the reproductive systems of legumes is determined by several considerations: First, to ensure efficient seed reproduction of species cultivated outside the range of commercially valuable species - food, medicinal, decorative; second, to protect rare and endangered species; third, to estimate the risk of adventitious species habitualisation in local plant communities. The assessment of disorders in the plant reproduction is of particular importance for the analysis of adaptive capacity, because it is impossible to address such disorders by agronomic or environment protection measures.

Evaluation of the reproductive system of a species includes a set of interrelated and interdependent indicators and the system of reproduction depends on it directly or indirectly. The most important among them is the life form, the formation of vegetative and generative organs, the availability and effectiveness of vegetative propagation, especially embryogenesis and fertilization, the formation of the elements of seed production and the factors determining its decline, the quality of seeds.

Materials and methods
We have studied the characteristics of the reproductive biology of legumes, which include the genera Amoria (A. fragifera L.), Roskov, (A. montana L.), Sojak, (A. repens
L. C. Presl.), Astragalus (A. cornutus Pall., A. karelinianus M. Pop., A. wolgensis Bunge, A. silvisteppaceus Knjasev sp. nov.), Chrysaspis (C. spadicecea L. Greene), Glycyrrhiza (G. glabra L., G. korshiskyi Grig., G. uralensis Fisch.), Melilotoides (M. platycarpos L. Sojak), Trifolium (T. trichocephalum Bieb., T. pannonucum Jack., T. pratense L., T. arvense L.) and Trigonella (T. foenum-graecum L., T. caerulea L.) being introduced in the botanical gardens and taken from natural populations in the Urals. We have determined the indicants of plant life forms, the effectiveness of vegetative propagation, pollination system and the advancement of the phenol phases by the introduced species. The real and potential seed production, seed production rate, sowing quality of seed germination and vigor were also assessed. Embryological analysis was performed by light and fluorescent microscopy (microscope LEICA DM 2500 B). The productive status of male and female systems was defined through pollen grains and ovules sterility, the structural features of anther wall, the development of stigma surface, the mutual arrangement of the androecium and gynoecium elements and the effectiveness of pollination. Sterile pollen grains were determined by the acetokarmin method and after staining with IKI to detect starch [1]. The structure and level of sterilization of ovules was determined after their clearing and maceration in etilsalitsilate [2]. The structure of the reproductive system and the effectiveness of pollination were studied by fluorescence microscopy by aniline blue [3]. Statistical processing of the material was performed using the programme Statistika 6.0.

Results and Discussion
Among the species studied both annuals and perennials are presented. Annual species are herbaceous monocarpics with a taproot. Perennial species are polycarpous herbs, shrubs, and semishrubs. Reproductive strategies characteristic for the species studied were determined. The first strategy is a combination of active vegetative and seed propagation, which is characterized by high rates of seed production by laying a large number of ovules, and a complex pollination system (Amoria repens). The second is a combination of active vegetative propagation and unstable propagation of seed (species of Glycyrrhiza, Amoria fragifera). The third is a combination of limited and ineffective vegetative reproduction and unstable system of seed propagation (perennial species Trifolium, Melilotoides, Astragalus). The fourth is the reproductive strategy of annual species, which is based solely on seed propagation the effectiveness of which depends on a complex system of pollination (annual species of Trigonella, Chrysaspis and Trifolium). The most important indication of the introduction and adaptation success is the full advancement through the plant life cycle. We recorded the dates and the duration of phenological phases. The study of the phenology of Trigonella foenum-graecum, Trifolium trichocephalum, Glycyrrhiza glabra, G. korshiskyi, G. uralensis shows that the length of the growing season and the duration of phenological phases change compared with the natural habitats. With the introduction to the Middle Urals the starting dates of budding and flowering are significantly delayed and the stage of bud formation and development is longer. In hay fenugreek the flowering period is significantly lengthened. However, all the main stages are performed successfully enough and in favorable years, the life cycle is completed.
The effectiveness of vegetative reproduction in perennial species is not the same. This is determined by the nature of underground bodies, the presence of specialized organs of vegetative spreading and reproduction. For example, a vegetative mobile species is licorice with strong creeping rhizome, as well as white clover and strawberry clover with long creeping shoots, rooting at the nodes on the ground, which provide not only the active growth and easy transition to particles independent existence, but also their profound rejuvenation. Species with a short rhizome, such as red clover have less effective vegetative reproduction. Caudex of mountain clover can provide only a senile participation that extends the generative phase of the individual, but does not rejuvenate.

The type of generative shoots is crucial to the evaluation of bean seed propagation. Legumes form simple axillary inflorescences of two types - the raceme and capitulumous raceme. In species with weak branching generative shoots (Amoria montana, Trifolium trichocephalum, T. pannonicum) flowering is short term. In species with highly branched generative shoots (Amoria repens, species of Glycyrrhiza and Melilotoides platycarpos), flowering period is very prolonged.

Microsporogenesis and male gametophyte development in all species studied are not significantly disturbed. As a rule, the level of sterilization is low, not significantly different from the sterilization level in natural habitats and it cannot have a significant impact on the efficiency of fertilization. Some individuals of Trigonella caerulea and Trifolium trichocephalum under introduction develop increased variability in the size of pollen grains. Along with the fertile grains of normal size, there are small and hypertrophied large pollen grains, which are sterilized at different stages of development. It is known that a number of external factors may influence the course of cytological processes during the formation of pollen grains. These include the intensity of illumination, nutrition and water conditions, low and high temperature. The external influence is strongly manifested in introduction due to the changes in habitat conditions and together with some increase of male gametophyte sterilization it is the reflection of plant response to a complex of new factors.

As shown by our study [4], one of the main factors limiting seed reproduction in the species of legumes studied is sterilized ovules. The ovary of annual and perennial species contains different number of ovules. White clover may have 2 (some types of clover, fenugreek blue), some licorice species from 2 to 6, Melilotoides platycarpos - 6-9, Trigonella foenum-graecum - 5-13, Astragalus cornutus - 16-25, A. karelinianus - 7-25, A. wolgensis - 21-35. Most of these ovules are sterilized before the start of flowering. The percentage of aberrant ovules in annual species, on the average, is 79% in Trifolium arvense, 50% in Chrysaspis spadicea, 18%, in Trigonella caerulea and 50% in T. foenum-graecum. In perennial species, this index is the lowest in Melilotoides platycarpos - 16% and the highest level of sterilization is noted for Amoria montana - 58% and Astragalus cornutus - 64%, A. silvisteppaceus - not less than 50%.

It is known that the death of a certain number of ovules is due to the laws of the species reproductive biology. There are general points, which specify that, on the average, for annuals the number of fertile ovules should be 85%, and for perennials - 50% [5]. Of course, genotypic characteristics do determine the interspecific and intraspecific variations in the degree of sterilization. However, attention is drawn to
the fact that the level of sterilization for plow clover, chestnut clover and fenugreek in the Urals is well above the average level for the annuals.

In the Middle Urals after the flower unfurling the fertility of the ovules without fertilization is preserved in *Trifolium arvense* for 2-3 days, *T. trichocephalum* - 3-4 days, *T. pratense* and *T. pannonicum* - 5-7 days, *Amoria montana* - 4-5 days, *Melilotoides platycarpos* - 6-7 days, *Trigonella caerulea* - 3-6, and *T. foenum-graecum* - 3-5 days. The prolonged flowering of entomophilous species is a factor in adaptation. Morphologically, the flowers papilionaceous, typical for this family, is the result of specialization in cross-pollination and a classic example of entomophilous flowers.

Our study shows that the genera *Amoria, Astragalus, Chrysaspis, Melilotoides, Trifolium, Trigonella* demonstrate a wide interspecific and intraspecific polymorphism of pollination systems. Some of them are obligate cross-pollinators, obligate self-pollinators and the species with complex systems that combine different types of cross-and self-pollination.

For example, certain species of perennial clover (clover pannonian, red clover from wild populations, clover trichocephalum), some species licorice and fenugreek plathycarpos are obligate cross-pollinators. In the entomophilous flowers of legumes there is a complex system of self-incompatibility [6], which includes, inter alia, the structure of the stigma surface, the need of tripping, filaments that are shorter than the column of pistil, which prevents the penetration of its own pollen on the stigma of the pistil in the cracking of the anthers. However, under experimental conditions, artificial self-pollination produced low (0.1 - 0.9%), but positive results. This suggests that in natural conditions geitonogamy is possible, although it does not contribute significantly to the reproduction of the population.

Autotripping, elongation of filaments and germination of pollen tubes through the anther wall facilitate self-pollination. Cultivated clover and fenugreek are obligate self-pollinators among the species studied. In the Middle Urals hay fenugreek significantly decreases the linear dimensions of the flower - the mean value of 7.1 mm (within its habitat the length of the flower is 13 - 19 mm), the color of the corolla has become less bright, it is whitish-green. The change of the morphological parameters of the flower can be considered as an element of the system dynamics towards autogamous pollination associated with plant adaptation to new environmental conditions, and possible optional autogamy.

Annual species of clover, white clover and blue fenugreek have a complex system of pollination, which includes both xenogamy and contact and gravitational autogamy. However, the effectiveness of different pollination types for reproduction is not the same, under normal conditions, as a rule, cross-pollination is prevalent. The combination of different pollination types is observed not only in different individuals of a species, but also within the same inflorescence.

Clove chestnut blossoms exhibit clear zoning with respect to the type of pollination. Within a single flower there are often signs of two or even three types of pollination in different combinations, which shows the great potential of the system to use the type of pollination, which will be beneficial from the genetic (under favorable conditions) or environmental (if cross-pollination is not possible) points of view.

White clover and forest-steppe *Astragalus* exhibit the substitute, protective character of autogamy. The study identified individuals of these species with
abnormally developed flowers, including the undeveloped stigmas and reduced columns. Stigma surface of pistils is absent, making cross-pollination or autogamy gravity impossible

Nevertheless, pollination occurred by the ingrowth of pollen tubes directly into the tissue of the column. The undoubted fact is that to achieve the final goal of pollination, i.e. fertilization, these species use the type of pollination, which is possible due to the effects of certain environmental factors and genetic status of the individual. At the same time, it should be noted that the structure of pollination and the contribution of the different types of pollination, as well as their implementation, reliably exhibit species-specific traits.

Parameter estimation of seed production has identified some general patterns in the species studied. First, there is high potential seed productivity because of producing a large number of generative elements. Second, there is a significant reduction in seed production, primarily due to the sterilization of the female gametophyte. This gives a very low rate of seed production at high absolute numbers of mature seeds formed. Third, there are very high levels of intraspecific variation in these characteristics as well as significant differences characteristic of the same species in different years.

For example, the analysis of seed production elements in three species of licorice gives an indication that a significant reduction in seed production continues after flowering. There is a degeneration of the whole of flowers and inflorescences. Korzhinsky licorice forms 19 to 40 flower buds, after fertilization 6-15 pods develop, from which matures only from 3 to 14 pods per collective fruit/stem. On the average, the plant produces from 2 to 16 inflorescences, which at the time of fruiting produce 2 - 12 aggregate fruits [7].

Thus, seed production is reduced considerably. The ratio of seed production in different samples of Korzhinsky licorice from the Southern Urals is from 0.5% to 27.6%. The highest and lowest rates of seed germination in different years are characteristic of Korzhinsky licorice - 33% and 11% respectively. On the average, the figure for Korzhinsky licorice is 22.5%, for liquorice - 15%, for Ural licorice - 19.2%. The study of seed production reveals that blue fenugreek showed significant ecological plasticity, which is manifested in the high qualities of crop seeds (germination rate - 82.5%) and high ratio of seed production (84%). This indicates the successful adaptation of species to new environments.

Hay fenugreek is characterized by the high percentage of germination (from 74 to 86%) and germination energy. But at the same time, low rate of productivity (0.35) indicates low seed renewability. The comparison of seed production shows that productivity factor of strawberry clover is reduced in the Middle Urals (38-44%) compared with similar data from the habitat in Dagestan (55.3%). At the same time, the ratio of fruiting increases in the Middle Urals at 1.2-1.3 and in Dagestan at 0.9. This can be explained by the fact that exotic species decrease the number of flowers in which both ovules form mature seeds. Because the Middle Urals forms the northern boundary of the species, we can assume that the decrease in seed production against the increased rate of fruiting is the result of strengthening the role of self-pollination.

Thus, the analysis of embryological and some others indicators associated with reproduction revealed the characteristic features of legume breeding. In addition to
traditional indicators of seed production and seed quality, sterilization of the female gametophyte is a very important indicator of the condition of seed multiplication system in all species. In our opinion, the high level of sterilization of the female gametophyte which is almost a universal sign for the members of this family is a limiting factor in embryonic development. As shown by our studies, an important factor in adaptability of some species of clover, Astragalus and fenugreek, besides the condition of the female gametophyte, is the potential ability to change the type of cross-pollination to self-pollination and the complex system of pollination.

For all the species studied, the express methods for assessing the condition of the female gametophyte are suggested. This allows rapid assessment of the reproductive potential of plants, which is especially important for carrying out environmental protection of rare and endangered species, the populations of which are on the verge of extinction.

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References