

The role of polygamy in *Salix myrsinifolia* expansion

Paweł Mirski

In the course of evolution Angiosperms developed various sexual systems – starting from hermaphroditism through many intermediate stages to dioecy, which is defined as the presence of male and female individuals in one population. In Angiosperms sex can be determined both by genetics (sex chromosomes, single- or multilocus), as well as environmental factors (habitat and climate conditions and its changes, parasites and others). The variability and cooperation of different sex determination mechanisms in plants makes them very flexible in adjusting their sexual systems to environmental conditions. Studying this plasticity and its determinants, we are able to assess plants' adaptive potential and colonization abilities. In this context, polygamy can serve as an especially interesting sexual system, in which, apart from males and females, populations also consist of hermaphrodites (bisexuals). Such defined polygamy can also be referred to subdioecy and trioecy. This phenomenon is one of the latest stages of dioecy evolution, and partly combines advantages connected to the presence of separate male and female individuals, which enables outcrossing, but also the presence of hermaphrodites, which, in contrast, enables seed set due to autogamy. Polygamy is a rather rare sexual system, which is found in 3.6% of Angiosperms, including the species which have already reached dioecy, but at least in parts of their geographic range are characterised by polygamy. Such species include: *Mercurialis annua*, *Acer negundo* and *Salix myrsinifolia*, which was the object of my study.

Dark-leaved willow *Salix myrsinifolia* is a shrub or a small tree of Eurosiberian, boreal-montane distribution. The south-western border of *S. myrsinifolia*'s geographic range runs through the north-eastern Poland, while it currently expands southward and westward across the country. Geographic expansion is accompanied by the frequent colonization of anthropogenic habitats. Dark-leaved willow is considered dioecious, similarly to the great majority of species in the *Salix* genus. However, Faliński (1998)¹ found that across

¹ Faliński J. B. 1998. Androgyny of individuals and polygamy in the populations of *Salix myrsinifolia* Salisb. in the south-western part of its geographical range (NE-Poland). *Perspectives in Plant Ecology, Evolution and Systematics* 1/2: 238–266

north-eastern Poland it commonly shows a polygamous sexual system. It is manifested by the occurrence of three sex-morphs in a population – individuals with an inflorescence comprised of both male and female flowers (hermaphrodite individuals), together with individuals having exclusively male inflorescences and individuals having exclusively female inflorescences. Faliński also found that the frequency of hermaphrodites increased together with the ruderalization of habitats and also increased in time (observations done on marked individuals in the Biebrza Valley and near Białystok, Poland). Moreover, hermaphrodite individuals were described by him as reaching greater size and characterized by higher growth and fertility. I have done a literature overview on exceptions from dioecy in *Salix* genus, showing that at least 18 different willow species (within a genus comprised of 330-500 species) are capable of some flexibility of sexual systems. However, this phenomenon is the most pronounced in *S. myrsinifolia* and accompanies its geographic expansion. This fact prompted me to focus my study around the main hypothesis, that the change of sexual system from dioecious to polygamous is an adaptation to the geographic expansion of this species. I have analysed relationship between returning to the polygamous sexual system and geographic expansion and put forward ten specific hypothesis. First, I wanted to confirm the main hypothesis by answering the question of whether the presences of polygamy is significantly connected to the secondary part of the geographic range? I assumed the frequency of hermaphrodites in populations inside the secondary range is higher than inside the primary range (h1). Then, I wanted to verify Faliński's suggestion about the higher competitive leverage of hermaphrodites over male and female individuals. I assumed that hermaphrodite individuals are characterized by higher competitive and reproductive potential over males and females. Their competitiveness should be manifested in rapid growth and greater size (h2.1), higher number of inflorescences, greater size of inflorescences and higher number of flowers per inflorescence (h2.2) as well as lesser susceptibility to herbivory than males (h2.3). Moreover, hermaphrodites should be more vital and show greater survivorship (h2.4). Also, I wanted to confirm that hermaphrodite individuals are more common on anthropogenic than natural habitats and on highly afforested areas, which could work as a barrier to its pollinators (h3). I also assumed that sex structure is variable both in time and space (h4). The frequency of hermaphrodite individuals should be influenced by the ratio of males and females, latitude (expansion follows a north-south gradient), and by the character of the local landscape (individuals of different sexes can show different landscape requirements) (h5). Moreover, taking into account Faliński's (1998) finding about the ability

of *S. myrsinifolia* to change its sex, I assumed that in black-leaved willow the sex is labile and inside the secondary range it should most often turn hermaphrodite (h6). I thought that the variability of polygamy in studied parts of the geographic range should be reflected by the spatial variability of species' genetic structure. I expected genetic variability to be high on the species level, because it is polyploid (hexaploid), it shows high reproductive potential, and is able to hybridize with other willow species. However, I expected genetic variation to be significantly higher in dioecious than in trioecious populations, because of obligatory cross-pollination in the first one and high probability of autogamy in the second (h8). I also hypothesized that hermaphrodites should differ from males and females by ploidy level or constitute a genetically separated group (h9). Finally, I assumed that polygamy in relation to dioecy is a secondary, not ancestral feature (h10). If it proved true, than it might confirm that change of sexual system is in the case of this species an evolutionary adaptation.

In order to verify the hypothesis stated above, in 2010-2011 I repeated observations of sex structure in 30 populations of *S. myrsinifolia* (16 in the secondary range, 14 in the primary) that were previously studied by Faliński in 1995 and 2001. Populations were distributed across a north-south gradient (in parallel to the expansion front) in north-eastern Poland (secondary range) and Lithuania together with Suwałki Region (primary range). To study the constancy of sex and chosen features of each sex I also continued Faliński's observations on 500 marked individuals in the Biebrza Valley. In both cases, during the flowering season I measured the height and diameter of individuals, I identified their sex, estimated the number of inflorescences and vitality of individuals (based on the ratio of dead branches) and noted signs of herbivory. Moreover, in a population on Białowieża Clearing I randomly picked 20 inflorescences from 20 individuals of each sex, counted the number of flowers, and measured the length of the inflorescence. The location of each population was registered with a GPS device. Obtained coordinates were used to assess the impact of ecosystem types on the sex structure of populations using Corine Land Cover database and GIS software, in which I calculated the area of each land cover type in a radius of 1 km and 5.5 km around populations. A sample of 40 individuals of different sexes from eight populations in the secondary part of the range was used to measure the content of nuclear DNA with flow cytometry and to estimate the ploidy level. From each population I collected leaves from not more than 20 individuals for the analysis of genetic structure. Then the laboratory analysis were carried out using three polymorphic markers of plastid DNA (*trnL*, *trnT-trnL*, *accD-psaI*) and nuclear DNA (AFLP method) in order to

assess the genetic variability of the species. In order to follow the possible expansion history of the species, I conducted the modelling of changes in species range over the last three centuries. I used the maximum entropy method, un-correlated bioclimatic variables (annual mean temperature, mean diurnal temperature range, isothermality, max temperature of the warmest month, temperature annual range, annual precipitation, precipitation seasonality) and species occurrence records from available databases.

Among thirty studied populations, 18 showed polygamous and 12 dioecious sexual system. Most of polygamous populations (81%) were found in the secondary part of the range, and hermaphrodite individuals were more frequent in the secondary (21% on av.) than the primary (3% on av.) part of the range. This proved the first hypothesis (h1), which I found fundamental for further investigation.

Although I managed to show the existence of trimorphism in polygamous populations, hermaphrodite individuals were intermediate between males and females in terms of: size, inflorescence length and number of flowers in the inflorescence. Tested traits did not prove the hypothesis on the higher competitive abilities of hermaphrodites during geographic expansion (h2.1, h2.2, h2.3, 2.4).

I did not confirm the hypothesis on the more frequent occurrence of polygamous populations on anthropogenic habitats and those isolated by woodland ecosystems (h3). It cannot be excluded that in the case of the latter hypothesis, the methods used were not sensitive enough, or in fact there is no relation between landscape factors and sexual system in this species, although Faliński reported the relationship with the ruderalization of habitats.

Observation of sex structure over a dozen years confirmed the hypothesis on the variability of sex structure in time and space (h4). The domination of female individuals (55%) in the primary range decreased to resemble more an equilibrium state between the male to female ratio (43% vs 35%) in the secondary part of the range. Changes in the share of hermaphrodites were the reverse, while the frequency of males did not differ significantly in time and space. Change in time was most pronounced in the case of hermaphrodites and showed two trends: a marked increase in the secondary part of the range, and a slow decrease in the primary part of the range. The ratio of hermaphrodites was positively affected by population size and negatively affected by latitude and the ratio of infrastructure in the landscape. The impact of the last two factors agreed

with hypothesis h5, but the negative trend of infrastructure was surprising since Faliński showed the opposite – that ruderalization positively affects the ratio of this sex. In agreement with findings by Faliński, I confirmed the lability of sex in *S. myrsinifolia* (h6). In the population of marked individuals (Grobla Honczarowska) as many as 12% of all individuals changed their sex at least once during 18 years. Most changes were towards hermaphroditism (67%), and much rarer to female (19%) or male sex (14%). Interestingly, sex change occurred much more often in dying individuals (just as in the case of other woody species – *Acer rufinerve*).

Molecular studies showed, contrary to expectations (h7), that genetic variation was at a relatively moderate level (mean per cent of polymorphic loci = $32.8 \pm 3.2\%$, Nei genetic diversity index = 0.117), which was slightly less or comparable to the results of similar studies on other European willows). However, in accordance with the next hypothesis, the genetic diversity was higher in dioecious than in polygamous populations, which was confirmed by significant differences in Nei's index (0.119 vs. 0.116) and the genetic divergence index (1.17 vs. 1.02). In the course of plastid DNA analysis, 10 haplotypes were identified: 6 frequent ones were common for dioecious and polygamous populations, while 4 unique ones were found only in dioecious populations inside the primary range, which also confirms the h8 hypothesis. Furthermore, I found that the parameters of nucleotide and plastid diversity and the genetic divergence index were significantly correlated with latitude – decreased, together with the expansion front.

Individuals of hermaphrodite sex did not differ from others in terms of ploidy. All investigated individuals, regardless of sex, had similar values of nuclear DNA content (2.16 pg/2C on av.), which points to hexaploidy. However, I found that 89% of all hermaphrodites represented one of the dominant haplotypes (I describe it as a colonizer-haplotype), whose frequency significantly increased in a north-south gradient. This may point to a genetic determination mechanism of hermaphroditism, as was assumed (h9).

Haplotypes represented by hermaphrodite individuals are only a restricted subset of all haplotypes found in dioecious populations. This fact suggests that the polygamy phenomenon is secondary to dioecy (h10), and the polygamous sexual system is a drawback to the previous evolutionary stage, which may be an adaptation to conditions on the edge of the species range.

Prediction modelling of change in species range showed that *S. myrsinifolia*'s range gradually expanded southwards in the last 200 – 250 years, with a velocity of around 80 km/100 years. However, further predicted changes suggest a reverse trend of shrinking the range and withdrawal of this species from the studied area at the end of the 21st century.

The results of my studies showed that *S. myrsinifolia*'s sexual system (and potentially in others Angiosperms too) can undergo changes that are the effect of adaptation to environmental conditions. In the case of dioecious species, such as the one studied, the return to a more flexible sexual system like polygamy may be an adaptive mechanism, facilitating the colonization of new habitats or geographic expansion. The plasticity of sexual systems in Angiosperms can be considered as an important evolutionary strategy. In the case of *S. myrsinifolia* this plasticity is manifested in a change of sexual system in the course of geographic expansion. The phenomenon of polygamy in *S. myrsinifolia* is favoured in the initial stages of population development (colonization and expansion) thanks to the ability of single hermaphrodites to reproduce generatively by autogamy. In the longer perspective, such a process may lead to unfavourable genetic and ecological effects. Therefore, during the stage of population stabilization it seems more advantageous to limit the autogamy process by the elimination of hermaphroditism and a return to dioecy.