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Paweł Mirski

Exceptions from dioecy and sex lability in genus *Salix*

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Abstract: Bisexualism (and its derivatives) is a rare exception in species which already acquired the dioecious breeding system. Although it occurs in some tree species, it is still rather uncommon, except for willows, in which bisexualism occurs in at least 18 species. In most willows such unusual individuals are subdioecious, hermaphroditic or monoecious, or produce intersexes (staminate flowers transform into pistillate flowers or the other way round). The frequency of non-dioecy can vary from a single individual to whole populations, but is in principle rare. Its possible causes are both environmental and genetic. In *Salix* sex lability is known to be affected by parasites and anthropogenic habitat change. In interspecific willow crosses there are numerous exceptions from dioecy that are mostly caused by polyploidization. Since sex determination in willows is genetic, environmental factors are expected to determine exclusively phenotypic sex traits.

Additional key words: hermaphroditism, polygamy, sex lability, subdioecy, willow

Address: University in Białystok, Institute of Biology, Świerkowa 20B, 15-950 Białystok, Poland,
e-mail:p.mirski@uwb.edu.pl

Introduction

The occurrence of monoecy and bisexualism in dioecious trees and shrubs is highly uncommon. They were observed in ca. 0.1% of individuals in dioecious plant populations (Rottenberg 1998), for example in genus *Taxus* (Iszkuło and Jasińska 2004), *Acer* (Mędrzycki et al. 2006), *Rhamnus* and *Poplar* (Rottenberg 2000).

Willows are widely thought to represent a strictly dioecious breeding system, but some exceptions from this principle have been reported since the 19th century. In this paper possible sexual states in genus *Salix* are discussed, in order to describe its sexual plasticity. A list of almost twenty species showing unusual sexual characters is also presented, to document the variety of exceptions from dioecy in *Salix*. Mechanisms of sex determination and factors affecting sex change are discussed in the light of the most recent knowledge on this subject. The “Web of Knowledge” database (accessed February 2013) was used to search for papers dealing with this issue. Keywords like “sex”, “hermaphroditism”, “subdioecy”, “polygamy”, “lability”, were used together with “willow” and “Salix” in the query. Papers that match the topic were selected for the review and also the literature cited therein. Unpublished observations by author and a colleague from same department on three willow species were also included in the review.

Issues related to sex determination were discussed also in reference to poplars as a sister genus in the same Salicaceae family, because of the lack of knowledge for *Salix* genus only.

Exceptions from dioecy and its occurrence in willows

A significant number of willows turn to show some exceptions from strictly dioecious condition. These exceptions include significant changes in flower morphology, such as developing both male and female reproductive units on the same flower (hermaphroditic and intersexual forms) (Smith 1940). However, the most common exception is presence of both male and female flowers on one individual (Smith 1940, Faliński 1998). In this case a number of different sexual systems can be distinguished. If all catkins comprise both male and female flowers, they are simply monoecious. If an individual develops two types of catkins: one with both male and female flowers and the other with only male flowers, it is androdioecious (or gynodioecious when female catkins occur together with bisexual catkins) (Beentje 2010). When all the three types of catkins (male, female and bisexual) are present on one individual, it is subdioecious (trimonoecious) (Dellaporta and Calderon-Urrea 1993). Similarly, on the population level the term subdioecy refers to the situation where female, male and bisexual (or hermaphroditic) individuals are all functioning in one population (Harder and Barret 2006).

There are about 18 willow species showing exceptions from dioecy. Thirteen of these species are known to occur in natural conditions and three were obtained experimentally or under the impact of artificial factors, like transplantation. Two were found in both natural and experimental set-ups. The frequency of exceptions from dioecy varies but generally remained low (Table 1). There exist whole populations of bisexual individuals, but they are very rare (Table 1). Only one species has been known to regularly function as a hermaphrodite. It is the neotropical willow *Salix martiana*, which is capable of producing male, female and hermaphrodite flowers. All flower types can be found on a single catkin (Rohwer and Kubitzki 1984). A different situation was found in *Salix acmophylla*, which is generally dioecious, but in one population all individuals were bisexual (Rottenberg 2007). The remaining examples presented in this paper do not refer to whole populations. In most cases there was no precise data on the frequency of bisexuals and other exceptions (Table 1), but in general mostly single individuals showing atypical sexual condition have been reported. In some species the frequency of bisexuals showed high geographical variation between populations. The occurrence of the three sexual morphs (males, females and bisexuals) in one population (i.e. subdioecy) was studied in detail in *Salix myrsinifolia*. This boreal species is mainly dioecious, but on the edge of its range numerous populations consisting of up to 35% of bisexual individuals were observed (Faliński 1998).

Frequency of bisexualism is likely to be higher in interspecific crosses, but evidence is lacking. For example, in *S. exigua* and *S. eriocephala* crosses bisexuals comprised between 0% to nearly 30% of progeny (Mosseler and Zsuffa 1989). A high number of interspecific crosses (involving 15 willow species) leading to androgyny were obtained by Heslop-Harison (1924), but no data on its frequency are available.

In most cases non-dioecious plants produced bisexual catkins, which results in changing the sexual system of the plant to subdioecy. Other possible deviations are hermaphroditism or monoecy. The latter can take two different forms: separate male and female catkins on the same plant (i.e. *S. rosmarinifolia*, Table 1) or catkins with female and male flowers mixed (i.e. *S. planifolia*, Table 1). Another unusual examples in willows are intersexes or metamorphosans, first described by Smith (1940). Staminate flowers in these forms are in different stages of transformation to pistillate flowers, resembling both flower types (*S. padophylla, S. brachycarpa*, Table 1). Rarely the opposite sex reversal, from pistillate to staminate flowers, was observed (*S. pseudolapponum*, Table 1).

Sex determination in willows

Mechanisms of sex determination in plants can vary greatly from the autosomal to the XY-chromosome determination (Ainsworth 2000). Until lately little was known about sex determination in *Salix*. Some authors suspected that sex in this genus is genetically determined, with males being heterogametic, although sex chromosomes have never been found (Mosseler and Zsuffa 1989). Other authors showed that presence of sex chromosomes is unlikely in this genus and suggested a multilocus autosomal sex-determination system (Alstrom-Rapaport et al. 1997, 1998). This hypothesis was than supported by molecular studies (Gunter et al. 2003, Semerikov et al. 2003).

Sex determination is also poorly understood in poplars, members of the same family: Salicaceae. However quite recently an evidence for developing characteristics of a sex chromosome in this genus emerged (Yin et al. 2008). Before no genetic basis of sex determination has been found over the last century (McLetchie and Tuskan 1994). It has been suggested that several loci are interacting in an epistatic way and determine sex in *Populus* species, as concluded from a study on *Salix viminalis*. In this species, a gender-associated marker was found, which is linked to a nuclear gene and associated with femaleness (Alstrom-Rapaport et al. 1998). Another molecular studies on *S. viminalis* revealed more sex-linked

Table 1. Sexual systems found in genus *Salix*

Salix species	Bias type	Commonness	Conditions	Location	Author
arctica	Androdioecious	Single individual	Natural	Iceberg Lake, Colorado	Smith 1940
amygdaloides	Hermaphrodite	0–15,2% of intraspecific crosses	Experimental	No data	Mosseler & Zsuffa 1989
amygdaloides	Hermaphrodite	No data	Natural	Southern Ontario	Mosseler & Zsuffa 1989
discolor	Hermaphrodite	0–3,4% of intraspecific crosses	Experimental	No data	Mosseler & Zsuffa 1989
exigua	Hermaphrodite	0–12,5% of intraspecific crosses	Experimental	No data	Mosseler & Zsuffa 1989
exigua	Hermaphrodite	No data	Natural	Southern Ontario	Mosseler & Zsuffa 1989
lucida	Hermaphrodite	0–24,2% of intraspecific crosses	Experimental	No data	Mosseler & Zsuffa 1989
padophylla	Intersexes	Single individual	Natural	Fish Creek, Colorado	Smith 1940
brachycarpa	Intersexes	Single individual	Natural	Poudre Lake, Colorado	Smith 1940
pseudolapponum	Intersexes	Single individual	Natural	Poudre Lake, Colorado	Smith 1940
planifolia	Monoecious	Single individual	Natural	Estes Park, Colorado	Smith 1940
rosmarinifolia	Monoecious	Single individual	Transplanted	Suraż, Poland	Tałałaj unpubl.
aurita	Subdioecious	rare	Natural	Białowieża, Poland	Faliński 1998
martiana	Subdioecious	Whole population	Natural	Obidos, Brasil	Rohwer & Kubitzki 1984
acmophylla	Subdioecious	Whole population	Natural	Nahal Dishon, Israel	Rottenberg 2007
caprea	Subdioecious	rare	Natural	Białowieża, Poland	Faliński 1998
cinerea	Subdioecious	Single individual or rare	Natural	Białowieża, Poland	Faliński 1998, Mirski unpubl.
exigua	Subdioecious	Few individuals	Natural	Bank of Cache La Poudre, Colorado	Smith 1940
lasiandra	Subdioecious	Single individual	Natural	La Porte, Colorado	Smith 1940
myrsinifolia	Subdioecious	5–35% of individuals	Natural	North-East Poland	Faliński 1998, Mirski unpubl.
scoulenaria	Subdioecious	Single individual	Natural	Rist Canyon, Colorado	Smith 1940

markers associated with femaleness (Gunter et al. 2003, Semerikov et al. 2003). This fact strengthens the hypothesis that sex in this species is determined genetically by one or more autosomal loci. Furthermore, a genetic basis of sex determination was found in interspecific crosses of poplars (Yin et al. 2008). A gender-associated locus was identified in chromosome XIX, which may be in the process of transforming into a sex chromosome. Also, some evidence has been found that points to a ZW sex determination system in genus *Populus* (Yin et al. 2008).

Still environmental impact on sex determination of Salicaceae family members is considered in some papers. McLetchie and Tuskan (1994) suggested it is possible that gender in poplars is determined environmentally at an early zygote stage canalizing gender expression. Mosseler and Zsuffa (1989) concluded that in case of hermaphrodite willows there might be an environmental control over genetic mechanism responsible for the suppression of female sex expression in the male genotype.

Sex change and sex lability

The frequency of hermaphrodite or monoecious individuals in willows can be driven by several factors. One of them is polyploidization. A cytogenetic analysis revealed a complex nature of chromosome numbers in willows. While natural populations are usually diploid, tetraploid, and hexaploid, interspecific crosses can be also triploid, pentaploid, heptaploid and octoploid (Håkansson 1955). It has already been documented that occurrence of different sexual systems in the same species can result from different ploidy levels (Pannell et al. 2008). This is mostly associated with hybridization. Occurrence of bisexuals through this mechanism seems very probable since willows are known to cross easily and bisexuality in hybrids is observed quite often (Mosseler and Zsuffa 1989). A few cases of abnormalities in hybrid willows (triploids, pentaploids) were described by Smith (1940) and cases of dioecious hybrid willows and intersexes from pure line crosses of *S. caprea* were reported as well.

Apart from genetic mechanisms of gender determination, other mechanisms, like sex lability, play a role in deviations from dioecy. In willows this phenomenon is still poorly documented. Several willow species are known to change sex from male to female, as a result of parasitic castration caused by eriophyid mites (Heslop-Harrison 1924). Sex change occurs due to significant changes in metabolism of the plant, for example caused by environmental factors. Androgynous and monoecious forms of willows probably arise that way (Heslop-Harrison 1924). Other factors, such as anthropogenic habitat change, can also be of importance (Faliński 1998).

In *S. myrsinifolia* up to 5% of marked individuals changed sex from strictly males or females to bisexuals. Changes in the opposite direction occurred even less frequently (Faliński 1998, Mirski unpubl.). There are only limited data on how often sex changes can occur during an individual's life. In studies on marked individuals of *S. myrsinifolia* only a few percent of willows changed sex and most of them did it only once in fifteen years (Faliński 1998, Mirski unpubl.). On the other hand, bisexuality in the willow crosses from the experiment by Mosseler and Zsuffa (1989) lasted only one season and in the next year all of the hybrid progeny turned to males.

Labile sex expression was also reported from a few populations of *Populus deltoids* in New Mexico and both directions of sex change were observed. Female flowers were formed subsequently to male flowers, suggesting protoandrious hermaphroditism rather than sex change.

Conclusions

Exceptions from dioecy, such as monoecy, subdioecy and intersexualism occur in many *Salix* species and its interspecific crosses. On the basis of the gathered data it can be concluded that probably even more willow species are capable of reversing from a strictly dioecious condition to one of the non-dioecious states.

Occurrence of non-dioecious individuals in willow populations is generally very low, but in some cases they can constitute whole populations or at least significant part of a population.

Sex determination and sex change mechanisms are driven by genetic factors but sex expression is controlled by both environmental and genetic factors (mostly polyploidization).

Sex change in willows can be reversible and short-lasting (i.e. labile sex expression).

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References

Ainsworth C. 2000. Boys and Girls Come Out to Play: The Molecular Biology of Dioecious Plants. Annals of Botany 86: 211–221.

Alstrom-Rapaport C., Lascoux M., Gullberg U. 1997. Sex determination and sex ratios in the dioecious shrub *Salix viminalis*. Theoritical and Applied Genetics 94: 493–497

Alstrom-Rapaport C., Lascoux M.,. Wang Y.C., Roberts G., Tuskan G.A. 1998. Identification of a RAPD Marker Linked to Sex Determination in the Basket Willow (*Salix viminalis* L.). Journal of Heredity 89: 44–49.

Beentje H. 2010. The Kew Plant Glossary. Richmond Royal Botanic Gardens, Kew.

Dellaporta S.L., Calderon-Urrea A. 1993. Sex determination in flowering plants. Plant Cell 5: 1241–1251.

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

Gunter L.E., Roberts G.T., Lee K., Larimer F.W., Tuskan G.A. 2003. The development of two flanking SCAR markers linked to a sex determination locus in *Salix viminalis* L. Journal of Heredity 94: 185–189.

Håkansson A. 1955. Chromosome numbers and meiosis in certain salices. Hereditas 41: 454–482.

Harder L.D., Barrett C.H. 2006. Ecology and evolution of flowers. New York, Oxford University Press.

Heslop-Harrison J.W. 1924. Sex in the Salicaceae and its modification by Eriophid mites and other influences. The British Journal of Experimental Biology 1: 445–472.

Iszkuło G., Jasińska A.K. 2004. Variation in sex expression in Polish and Ukrainian populations of *Taxus baccata* L. Dendrobiology 52: 29–32.

Mędrzycki P., Kołaszewska B., Browiński P., 2006. Subdioecy in invasive populations of *Acer negundo* (*Aceraceae*) in eastern Poland. Polish Botanical Studies 22: 355–364.

Mosseler A., Zsuffa L. 1989. Sex expression and sex ratios in intra- and interspecific hybrid families of Salix L. Silvae Genetica 38: 12–17.

McLetchie D.N., Tuskan G.A. 1994. Gender determination in *Populus*. Norwegian Journal of Agriculture Science 18: 57–66.

Pannell J.R., Dorken M.E., Pujol B., Berjano R. 2008. Gender variation and transitions between sexual systems in *Mercurialis annua* (*Euphorbiaceae*). International Journal of Plant Science 169: 129–139.

Rohwer J., Kubitzki K. 1984. *Salix martiana*, a regularly hermaphrodite willow. Plant Systematics and Evolution 144: 99–101.

Rottenberg A. 1998. Sex ratio and gender stability in the dioecious plants of Israel. Botanical Journal of the Linnean Society 128: 137–148.

Rottenberg A. 2000. Fertility of exceptional bisexual individuals in four dioecious plant species. Sexual Plant Reproduction 12: 219–221.

Rottenberg A. 2007. Fertility and sexual structure in a polygamous willow population. Plant Systematics and Evolution 268: 257–260.

Semerikov V., Lagercrantz U., Tsarouhas V., Rönnberg-Wästljung A., Alström-Rapaport C., Lascoux M. 2003. Genetic mapping of sex-linked markers in *Salix viminalis* L. Heredity 91: 293–299.

Smith E.C. 1940. Sex expression in willows. Botanical Gazette 101: 851–861.

Yin T., DiFazio S.P., Lee G.E., Zhang X., Sewell M.M., Woolbright S.A., Allan G..J., Kelleher C.T., Douglas C.J.,Wang M., Tuskan G.A. 2008. Genome structure and emerging evidence of an incipient sex chromosome in *Populus*. Genome Research 18: 422–430.