Are hermaphrodites better adapted to the colonization process in trioecious populations of *Salix myrsinifolia*?

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Abstract

In trioecious plant populations, the role of hermaphroditism is often uncertain. We investigated the advantages of hermaphroditism in the dioecious shrub *Salix myrsinifolia*. The sex ratio of 30 *S. myrsinifolia* populations in northeastern Poland (secondary range) and Lithuania (primary range) was investigated in 2010–2011. Measures of reproductive (number of catkins, number of flowers in catkins) and vegetative traits (height, diameter, number of shoots, vitality) were taken and compared among sexual morphs. In two populations, measurements collected 14 years prior on marked individuals were used to determine the rate of changes in height, crown diameter and survivorship rates. We found trioecy mostly in the secondary part of the range with an average share of hermaphrodites reaching 21% in the trioecious populations. The sex ratio varied between populations, but tended mostly towards female domination. Several traits differed significantly among sexes. The characteristics of hermaphrodites were often intermediate between males and females and provided no evidence for the higher competitive abilities of hermaphrodites. We concluded that the possible gain of hermaphroditism in the colonization process is restricted to reproduction. We consider hermaphroditism in *S. myrsinifolia* as an equilibrium between the allocation of resources for growth and reproduction in unstable conditions on the margins of the range.

Keywords: subdioecy; polygamy; plant gender; size structure; sexual trimorphism; sex ratio

Introduction

Sexual dimorphism in plants is characterized not only by the production of androecia vs. gynoecia (primary sexual characters) but also by a range of other morphological differences (secondary sexual characters) among sexual morphs. Males and females can also vary in traits such as individual leaf and flower size, the number of flowers, resource allocation and many others ([1,2] and references therein). Moreover, both sexes can be differentially influenced by biotic conditions such as parasitism and herbivory, which affect their competitive ability [3]. The latter is especially important [4] and even considered a potential force in the evolution of separate sexes from hermaphroditism [5]. Furthermore, differences in resource allocation and reproductive morphology may drive opposite sexes to specialization in different habitats (sexual specialization) and results in the spatial segregation of sexes [6]. It is likely that the phenomenon of spatial segregation of sexes may also be a result of inter-sexual competition, which may impact plants more significantly than intra-sexual competition [7].

Size dimorphism is considered to be a result of different resource allocation in sexually dimorphic plants [8]. In general, females are considered to grow less due to a greater investment in reproduction, which was especially proven for woody plants [8–11]. Other factors, such as local mate competition, geitonogamy, and pollination vectors [12,13], can also influence the benefits of sex–size relations, depending on the species. Size itself positively correlates with flower production [14], the number of offspring [15] and survival probability [16,17] and therefore might serve as a good indicator of competitive abilities.

Sex-dependent resource allocation is one of the evolutionary forces through which dioecy evolves via the gynodioecy pathway [18]. Although the evolution of separate sexes in plants has many advantages [19], drawbacks to sexual systems involving hermaphrodites have been recorded in several tree genera, mostly in *Salix* [20] and *Acer* [21–23], as well as in *Taxus* [24], *Rhamnus* and *Populus* [25]. Why is this turnover favorable if it is a step back in the evolution of sexual systems? It appears that introducing hermaphrodite individuals to previously dioecious populations may be favored in unstable conditions, such as under skewed sex ratios [26], landscape fragmentation [27], lack of pollinators [28], significant habitat alteration [29], or when the opportunities for cross-fertilization are rare, e.g., under the

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colonization process [30]. In all of the mentioned situations (resulting in low species density or population connectivity issues), hermaphrodites increase the possibility of generative reproduction with the nearest individual of any sex providing both pollen and ovules. Moreover, allocation to both male and female functions may be profitable when pollen and seed production are limited by different resources or when the cost sharing of producing pollinator attraction is more effective for both organs together [30].

Hermaphrodites are often said to differ morphologically from unisexual individuals. Faliński [29] and Nanami et al. [31] reported that hermaphrodite individuals of woody species were significantly larger than males and females. Similarly, in an Australian herb, *Wurmbea dioica*, hermaphrodites were found to be significantly taller than other sexes but only in some years [32]. Moreover, Faliński [29] reported that bisexual catkins in *S. myrsinifolia* were significantly larger than in other sexes but showed no data confirming this observation. In *Sagittaria latifolia*, hermaphrodites differed from males by having larger ramets and producing more flowers [33]. In *W. dioica*, hermaphrodites produced significantly more flowers than males and females [34].

Because mates can be rare during colonization and the invasion of new areas, sexual systems such as asexuality and selfing may be favored, and a switch in sexual systems could be observed in the populations at the edge of a range or at the edge of an invasion. In sexually dimorphic populations under colonization or in meta-population conditions, hermaphroditism is likely to become common [34]. Self-compatible hermaphrodites are most likely to be found in populations when species density is low and when there is a lack of pollinators. In addition, they are adaptable in resource allocation between pollen and ovules or between the perfect and pistillate/staminate number of flowers [35].

Although the theoretical explanations of hermaphroditism benefits are well discussed (i.e. [26,28,30]), they are rarely studied in the field when three sex forms of one species are involved (i.e. [33]).

Salix myrsinifolia, a boreal Eurasian species, is regularly dioecious; however, on the margins of its geographical range, it produces bisexual catkins (containing male and female flowers in various patterns of both sexes partitioning on the catkin) [29]. We refer to this state as hermaphroditism, as "plants that have both male and female function but in separate flower" [36]. Populations found on the southwestern margins of this species range often involve three sexual morphs: males, females and hermaphrodites (in the sense described above) and are referred to further as trioecious [36]. The *S. myrsinifolia* range in Poland expanded in the XX century [37–39] and became more numerous, which was observed recently between 1987–2003 [40]. *Salix myrsinifolia* appeared commonly in locations where it was rare or absent a few decades ago and was even characterized by Faliński [40] as behaving "invasive". Most probably *S. myrsinifolia* is under slow range expansion and vast majority of Poland can be attributed to secondary range, where species distribution increases, number of population grows and different anthropogenic habitats are being inhabited. In contrary to primary range, where species is mostly noted in riparian habitats, in Poland it is most often inhabiting waste pits, ditches along roads and abandoned grasslands [29]. The border between Poland and Lithuania approximately coincides with the border between the primary (Baltic States, Fennoscandia and Northern Asia) and secondary parts (Poland) of the geographical range of *S. myrsinifolia*.

Faliński [29] noted that hermaphrodite individuals are characterized by faster growth and larger catkins. Male parts on bisexual catkins developed before female parts. Seed germinability did not differ between females and hermaphrodites. The sex of this willow showed some lability with approximately 5% of individuals changing their sex during the study. Moreover, Faliński concluded that trioecy in this species has recently become much more frequent in Poland, which together with its spatial occurrence, suggests that hermaphroditism may be an advantage during the colonization process (particularly at the southern borders of the range).

The aim of our study was to test whether hermaphrodite individuals in *S. myrsinifolia* are different from males and females according to their size, reproductive potential, survival rates and range of occupied habitat conditions that might facilitate colonization of new areas. We aim to test the following hypotheses:

(*i*) hermaphroditism in *S. myrsinifolia* is biased towards greater frequency in the secondary part of their geographical range;

(*ii*) hermaphrodites have better reproductive potential, which is manifested by a higher number of produced catkins, longer catkins and more flowers on catkins;

(*iii*) hermaphrodites grow faster and attain greater size than other sexes;

(*iv*) hermaphrodites are less likely to be susceptible to herbivory than males (similar to other willows [41,42]);

(*v*) vitality and survival rates of hermaphrodites are greater than that of at least one of the other sexes;

(*vi*) trioecy is more frequent in afforested (potentially isolated from pollinators) and anthropogenic habitats (according to Faliński [29]).

Material and methods

The field study was conducted in 2010–2011 at the southwestern margin of the *S. myrsinifolia* range in northeastern Poland and in the main parts of the range across central Lithuania. Populations of *S. myrsinifolia* (considered as cluster of individuals growing in generally uniform habitat) were searched for across 560 km of the south–north gradient following Faliński [29] (Fig. 1). In each of the 30 populations studied, all individuals were classified to one of three sex morphs: males, females or hermaphrodites. A single individual was concerned as a shrub distinctive in: architecture of branches, sex of the catkins (male, female or mixed), uniform leave sizes, color and morphology. Non-flowering individuals were impossible to distinguish their sex and were excluded from the study.

To verify the existence of trimorphism in this species and to test Faliński's [29] assumption on the more vigorous growth and better reproductive potential of hermaphrodites, we studied the frequency and size structure of the sexual
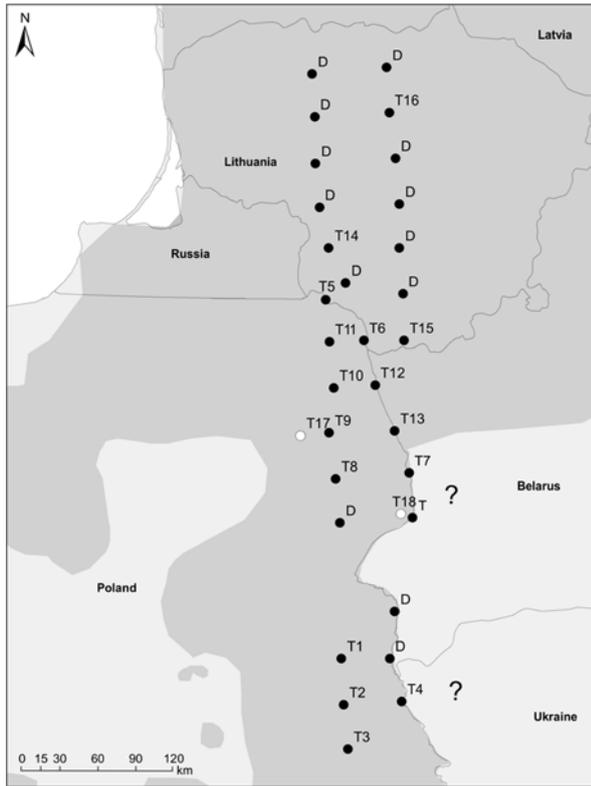
Fig. 1 *Salix myrsinifolia* study populations. The hatched background represents species range (according to [37–39], combined) with possible gaps (marked "?"). T – trioecious populations; D – dioecious populations; black dots – populations studied once in 2010 or 2011; white dots – populations where marked individuals were investigated in 1995 and 2010.

morphs of *S. myrsinifolia* populations. The following traits of individuals were measured in each population: height (m), crown diameter (m), number of shoots, number of catkins, and vitality. Height and crown diameter were measured with a scaled pole. Diameter was measured in two dimensions: first, the most broad measure, second, the perpendicular measure, and the two values were then averaged. Shoots were counted just above ground level. The number of catkins was estimated in classes as follows: less than 100, 101–1000, over 1000. Vitality was also estimated by the percentage of dead fragments of shoots and branches and ordered in the following classes: 5% or less, 6–25%, 26–50%, over 51%. In two large populations (T17 and T18; see Fig. 1) studied previously by Faliński [29], we measured catkin traits in 30 individuals (ten males, females and hermaphrodites) at the medium flowering stage, when the catkins were fully developed, but female ones were not fruiting yet and male ones did not already decayed. From each individual, 20 catkins were randomly selected. The length of the catkin was measured, and the number of male/female flowers was counted. In addition, in one of the two populations we measured the growth rate (Biebrza Valley, Poland, T17; see Fig. 1) of 224 individuals similarly studied in 1995 by Faliński [29]. After 14 years, individuals that survived were studied as in the previous study, and the differences in height and average crown diameter were calculated for each individual, and the

results were compared among sexes. In the same population, we studied the survivorship of males, females and hermaphrodites and expressed it as the rate of individuals of each sex that were still alive 14 years after marking. Additionally, to check if whole populations differed significantly from each other, we pooled the data from separate sexes and tested the possible traits (height, crown diameter, number of shoots, number of catkins, vitality) among the study populations.

To test additional environmental factors on the properties of individuals and sex ratio, population habitats were described and divided into groups: open/forested and natural/anthropogenic. We considered the *S. myrsinifolia* population open ($n = 19$ populations) if large trees constituted less than 25% and forested ($n = 7$) if they constituted more than 75% of the nearest surroundings (40 m radius). Populations in areas with afforestation between 25 and 75% were not distinct enough to include in comparisons. A population was considered natural ($n = 10$) if it was located in a natural depression or on lake edges, away from drainage ditches and roads, and together with natural or semi-natural vegetation such as alder forest, rushes, sedges or grasslands. Anthropogenic populations ($n = 12$) were located in dump pits or along drainage ditches and roads. Some populations shared both natural and anthropogenic conditions and were excluded from this analysis.

Signs of damage (debarking, shoots grazing) by large herbivores on each individual were noted to check if sex-biased herbivory occurs in this species. Then, the amount of damaged individuals among sex morphs was compared.

The comparisons of characteristics between the sexes were performed only for populations in which trioecy occurred, because the occurrence of sexual dimorphism in dioecious plants is already well known [2]. The significance of morphological differences between sexes in height, crown diameter, catkin length, growth rate and the number of flowers in catkins were tested using ANOVA. When significant differences were obtained, Tukey post-hoc tests were used to indicate the sex distinguishing from others. Differences between each studied trioecious population in the above mentioned characteristics were also done with ANOVA. Pearson and Spearman correlations were conducted for parametric and non-parametric tests, respectively. Differences in the number of catkins, viability and herbivory rate were tested with the Chi-square test assuming equal proportion results among the three sexes in expected values. Comparisons of the characteristics of individuals in open/forested and natural/anthropogenic populations were performed using a Mann–Whitney U test.

Results

Sex ratio

Sixteen out of the 30 study populations showed the trioecy phenomenon (Tab. 1), mostly (81%) in the southern part of the studied area (outside the primary geographic range) (Fig. 1). Non-flowering individuals were noted (constituting 0–20% of populations, 8% ±10% *SD* on average) but excluded from further sex ratio comparisons. Hermaphrodite individuals constituted 3–42% of the populations, with an

**Tab. 1** Sex ratio in *Salix myrsinifolia* trioecious populations studied.

Population number	Number of individuals	Proportion of females	Proportion of hermaphrodites	Proportion of males
1	20	0.35	0.35	0.30
2	20	0.45	0.25	0.30
3	9	0.22	0.11	0.67
4	21	0.38	0.33	0.29
5	21	0.55	0.12	0.53
6	32	0.52	0.26	0.23
7	12	0.42	0.42	0.17
8	10	0.50	0.20	0.10
9	11	0.64	0.09	0.18
10	30	0.55	0.32	0.14
11	37	0.52	0.12	0.36
12	28	0.27	0.42	0.31
13	29	0.38	0.03	0.59
14	19	0.53	0.11	0.37
15	30	0.35	0.15	0.50
16	27	0.62	0.04	0.35

average of 21% ±13% *SD*. The average ratio of hermaphrodites to males was 0.89 ±0.79 *SD* and varied widely between 0.05 and 2.5. The ratio of hermaphrodites to females was more stable and reached an average of 0.53 ±0.39 *SD*. The ratio between males and females reached 1.02 ±0.83 in all the study populations. In the trioecious populations, it reached an average of 0.91 ±0.71 *SD* and 1.06 ±0.94 *SD* in the dioecious populations, but the ratio largely varied in both groups. In the trioecious populations, females dominated over males in 68% of the populations and over hermaphrodites in 81% of the populations. In the dioecious populations, females dominated over males in 57% of the populations. Only in one population were hermaphrodites more common than males or females (Tab. 1, population No. 7). There was no correlation between the number of hermaphrodites and the number of individuals in a population ($R = -0.151$, $P = 0.577$) or with the male to female ratio ($R = -0.255$, $P = 0.340$). The number of catkins produced by each sex was distributed unevenly between the estimated quantity classes ($\chi^2_8 = 33.07$, $n = 330$, $P < 0.00001$). Most individuals produced 101–1000 catkins, but females predominated in the lowest class (less than 100 catkins; Fig. 2a). The number of catkins produced (grouped to classes) was significantly correlated with the height (Spearman rank correlation, $R = 0.239$, $P < 0.001$) and crown diameter of individuals (Spearman rank correlation, $R = 0.328$, $P < 0.001$). No such relationship was found with vitality ($R = -0.023$, $P = 0.672$).

The individuals of different sexes differed significantly from other sexes in the number of flowers in the catkin (ANOVA + Tukey HSD, $F = 427$, $P < 0.0001$) and catkin length (ANOVA + Tukey HSD, $F = 100$, $P < 0.0001$). Females produced the smallest number of flowers per catkin, and males produced almost twice as many flowers.

Bisexual catkins had slightly more flowers than female catkins (Fig. 3a). Each bisexual catkin contained an average of 55 ±37 *SD* male and 30 ±23 *SD* female flowers (Fig. 3b), which constitutes approximately 40% of the average number of flowers on both male and female catkins. The flowers had the highest density on male catkins (67 ±26 *SD* flowers/cm) and the lowest density on female catkins (18 ±5 *SD* flowers/cm), whereas bisexual catkins reached intermediate values (32 ±16 *SD*). The number of flowers of both sexes on bisexual catkins was significantly negatively correlated ($R = -0.326$, $P < 0.001$).
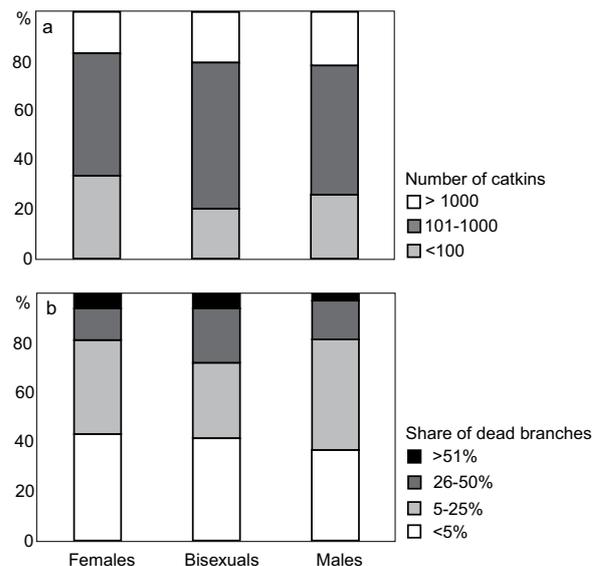
Fig. 2 Comparison of number of catkins produced (**a**) and vitality (**b**; represented by the percentage of dead branches) of each sex of *Salix myrsinifolia* in 16 analyzed trioecious populations.
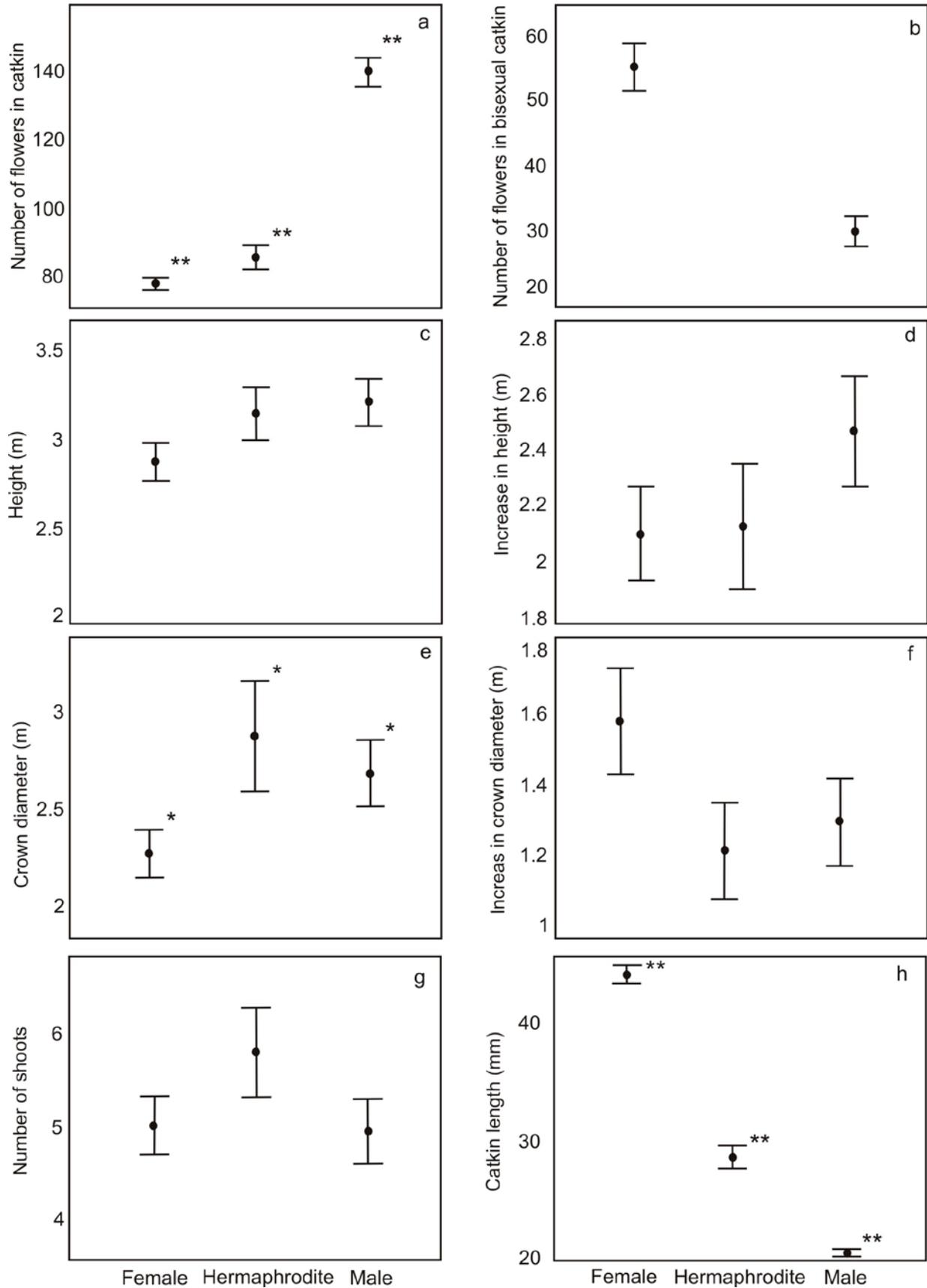
Fig. 3 Comparison of the *Salix myrsinifolia* sexes' mean values and standard errors of number of flowers in catkins of each sex (**a**) and number of male and female flowers in bisexual catkins (**b**), height (**c**) and its increase during 14 years (**d**), crown diameter (**e**) and its increase in during 14 years (**f**), number of shoots (**g**), catkin length (**h**). Results presented in plots a,b,h were measured in two populations (n = 600 catkins), plots **c,e,g,h,** in 16 trioecious populations (n = 356 individuals), plots **d,f** in one population of marked individuals (n = 224) in the 14-year period. Bars marked with "*" show significant difference in ANOVA test in particular feature among all sex morphs. Bars marked with "*" differed significantly from each other sex morph when tested with Tukey post-hoc test.

The survivorship of different sexes in the population of individually marked *S. myrsinifolia* did not differ significantly from the expected values during the 14 years of monitoring ($\chi^2_5 = 1.748$, $n = 500$, $P = 0.472$,) although the survival rates of females (41%) and hermaphrodites (42%) were smaller than that of males (48%).

Size structure

The hermaphrodites reached intermediate values between males and females in height and growth rate (Fig. 3c,d), whereas the crown diameter and growth rate were similar to males (Fig. 3e,f). The differences were significant in crown diameter (ANOVA, $F = 3.026$, $P < 0.0001$) but not in height (ANOVA, $F = 2.343$, $P = 0.098$). However, the differences in crown diameter were not significant between each sex when tested post-hoc using Tukey's HSD. The hermaphrodites slightly dominated over males and females in the number of shoots (Fig. 3g), but the value was not significant (ANOVA, $F = 1.207$, $P = 0.3$).

Regarding the growth rate, no significant difference was noted for height (ANOVA, $F = 1.168$, $P = 0.314$) or crown diameter (ANOVA, $F = 1.959$, $P = 0.150$), but the females showed the greatest crown diameter growth; males grew faster in terms of height (Fig. 3h). Significant differences among populations were found for all the tested variables: height (ANOVA, $F = 6.298$, $P < 0.0001$), crown diameter (ANOVA, $F = 8.072$, $P < 0.0001$) and number of shoots (ANOVA, $F_2 = 4.621$, $P < 0.0001$).

Impact of environmental conditions

The frequency of trioecy in *S. myrsinifolia* populations differed between natural and anthropogenic habitats (Mann–Whitney test, $U = 5500$, $Z = -2.441$, $P < 0.05$) but not between open and forested habitats (Mann–Whitney test, $U = 16\,500$, $Z = -0.496$, $P = 0.620$). There was a significant difference in vitality between individuals in open and forested populations (Mann–Whitney U test, $U = 6240$, $Z = -2.197$, $P < 0.05$) and the number of catkins produced (Mann–Whitney U test, $U = 6647$, $Z = -2.375$, $P < 0.05$). Differences were also noted for vitality (Mann–Whitney U test, $U = 10\,804$, $Z = -2.08$, $P < 0.05$) and height (Mann–Whitney U test, $U = 11\,172$, $Z = -2.234$, $P < 0.05$) between populations in natural and anthropogenic habitats. Individuals in the latter were taller and in better condition.

There was no difference in herbivore pressure among the sexes ($\chi^2_2 = 0.017$, $n = 236$, $P = 0.99$). In fact, individuals were debarked very evenly: 11–12% of shrubs of each sex showed signs of herbivory. Vitality was sex biased and significantly differed from an equal distribution among the sexes ($\chi^2_{11} = 34.3$, $n = 324$, $P < 0.001$). Females were observed to be in good condition more often than expected, which was in contrast to hermaphrodites (Fig. 2b).

Discussion

Trioecy (referred to also as polygamy or subdioecy in the literature) is not an exception in *Salix*, contrary to the former common opinion. It was found mostly in single individuals but sometimes included the entire populations

of at least 18 species [20]. We found this phenomenon common in *S. myrsinifolia*, especially on the borders of its geographical range. Moreover, we showed that sexual trimorphism exists in *S. myrsinifolia*, and the number of traits (crown diameter, number of shoots, catkin length, number of flowers in catkin) differed between the sexes. The traits of hermaphrodite individuals were mostly intermediate between males and females, and the assumed higher competitive abilities of hermaphrodites were not confirmed, based on the tested factors. However, the high percentage of hermaphrodites found in the populations in the southwestern range of distribution suggests possible advantage connected to this sexual state. No prevalence of hermaphrodites connected with faster growth, larger size or vitality was found in this species, contrary to the opinion of Faliński [29]. In *S. myrsinifolia*, both the height and diameter were lowest in female individuals, in which reproduction (production of both flowers and seeds) is most costly [8]. Our studies may support the hypothesis about a trade-off between growth and reproduction parameters. According to the trade-off hypothesis, female individuals are characterized by slower growth or smaller size due to the larger costs of reproduction, which is also the case in *S. myrsinifolia*. Leigh et al. [43] who summarized reproductive allocation in 41 dioecious species (including three subdioecious), found that in 39 of the species, the reproductive allocation of females was greater than males. Similar results were brought by Obeso, who found that in 20 out 23 species (both woody and herbaceous), female investment in reproduction was significantly greater [8].

Because no similar conclusions could be derived from this study on *S. myrsinifolia*, the possible gain of hermaphroditism is most likely restricted to reproduction. Sex morphs in *S. myrsinifolia* trioecious populations differed by traits connected with reproduction: size of inflorescences and the number of flowers in catkins. Our study showed hermaphrodites to have only slightly more flowers than females. The number of female flowers in catkins was considerably smaller than that of male flowers, which is not surprising considering the higher costs of producing female flowers [44]. On average, hermaphrodites produced about 40% of the number of flowers produced by both males and females. The catkins of hermaphrodites were intermediate in size and the number of flowers between males and females; in comparison to males, the catkins were larger, and in comparison to females, they had slightly more flowers. Because inflorescences (or catkins in this case) of larger size are more often visited by pollinators [45], the advantage of catkin size (floral display) may be crucial for reproduction, especially in fragmented populations when there is a shortage of pollinators.

Floral display size (in our case expressed as catkin length and the number of flowers on catkins) is important both for pollination by insects and wind. Floral display size contributes to the attraction of pollinators [43,46] in the case of both sexes, but in females it also increases the recipient area where pollen can be deposited. Thus, the size of the floral display influences both male (donors) and female (recipients) success but in a different way – in males, through the production of a higher numbers of flowers per catkin than in females; in females, through the production of larger/longer catkins. The

intermediate values of these parameters in hermaphrodites indicate that they can be visited more often by pollinators than males, and at the same time female flowers can be fertilized. The relationship between hermaphrodites and pollination was also considered by other authors, which suggests that pollinator abundance and pollen limitation play an important role in the maintenance of trioecy [47].

As willows are mostly dioecious (but see [20]), the hermaphrodite individuals in some species were not checked for selfing. It is probable that hermaphrodites in willows are self-compatible, and large pollinators such as bees or even the common Blue Tit *Cyanistes caeruleus* [48] may transfer pollen grains to stigmas between flowers on the same catkin. Such a transfer is facilitated in *Salix* because some species are both wind and insect pollinated (e.g., [49,50]). Wind pollination is facilitated in this genus by early flowering (before leaf development) and the release of pollen into the air. Flower morphology enables wind pollination – the perianth is absent, whereas the stamens and pistils outreach the catkin [51].

The androdioecious tree *Fraxinus lanuginose*, previously considered self-sterile, was revealed to be capable of selfing [52]. Moreover, selfing rates were higher in populations with low male frequency. Low male frequency was, however, correlated to the low density of individuals and influenced selfing by limited cross-pollination [52]. Similarly, other woody species, such as heterodichogamous *Acer mono* and *Juglans ailantifolia*, are quite prone to selfing [53,54], although they are endowed with mechanisms to avoid selfing.

Herbivory (at least by large mammals) was not biased by sex as it could be expected from studies on other willow species [41,42], therefore no clear advantage of any sex morph can be highlighted here. Also regardless of sex, individuals in populations overgrown by forest produced fewer catkins, so none of the sex morphs proved to be better resisting the light competition. Hermaphrodites showed however some habitat preference and grew more often in anthropogenic habitats, confirming the association of the ruderalisation of habitat and the incidence of trioecy in this species [29].

Although we did not find any advantage of hermaphrodites in the measured traits, it seems that their presence (sometimes with high frequency) on the margins of the geographic range is the best evidence of the benefit of this trait. Because *S. myrsinifolia* is mostly dioecious in central parts of the range (examination of herbarium collections

in [29]), the advantages concluded above are not universal and are restricted to the margins of the range. The other question is whether trioecy is a temporal phenomenon in *S. myrsinifolia*, connected with only some phases in population history (colonization) or whether it has become a new trait adapted to other climatic/environment conditions outside the primary geographic range. Simulated climatic changes weakly affected the differences in the traits of males and females in *S. myrsinifolia* [55]; thus, another sex probably did not directly result from different climatic conditions. It was most likely influenced by population demography. According to Yu and Lu [27], dioecious plants are sensitive to changes, especially in population size and structure, which can be a realistic issue in the colonization phase or under unusual conditions on the margins of the range, where populations are more fragmented and isolated. Many authors observed that individuals are often smaller and have lower rates of generative reproduction and more intense vegetative reproduction in marginal populations. In the first phase of population development, in the case when only one individual colonizes a new location (the founding individual), the production of progeny is impossible in unisexual individuals in contrast to hermaphrodites forming flowers of both sexes, which enables selfing. Thus, it seems that sex lability to hermaphroditism could be one of the important advantages of pioneer or expansive species (e.g., *Acer negundo* in Poland [23]). An experimental study on *Silene vulgaris* confirms that hermaphrodites can obtain better reproductive success than females when the population is isolated [56]. Bottlenecks acting during range expansion become a selective force for sexual systems in this scenario [57]. The presence of trioecy in the populations of *S. myrsinifolia* we studied may be strengthened in this way.

In conclusion, the hermaphrodites in *S. myrsinifolia* trioecious populations are only partly morphologically different from males or females. Contrary to our expectations, their characteristics were intermediate between males and females.

The basic evidence of their role in species expansion (the colonization of new areas) is the fact that they are present almost exclusively outside the areas of the main geographic range. It seems that the possibility to have three sex morphs could be one of the important characteristics of pioneer or expansive species. It still remains unclear whether this advantage is temporal or adaptive in a true sense.

Authors' contributions

The following declarations about authors' contributions to the research have been made: research designing, conducting field study, analyzing data, writing the manuscript: PM; research designing, partly writing the manuscript, advising: EB.

Competing interests

No competing interests have been declared.

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