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ALTITUDINAL VARIATION OF THE SEX RATIO AND SEGREGATION BY GENDER IN THE DIOECIOUS PLANT *MERCURIALIS PERENNIS* L. (EUPHORBIACEAE) IN SERBIA

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Abstract – The sex ratio is one of the most intriguing problems for evolutionary biologists. Spatio-temporal variation of male frequency in sexually dimorphic plant species, spatial segregation, and differential investment of the two sex functions have attracted much research interest. In this study, we examined altitudinal variation of the sex ratio and segregation by gender in *Mercurialis perennis* (dog's mercury), a dioecious anemophilous species with wide distribution. The eight studied populations from Serbia represented an altitudinal range of 196 to 1480 m. Sex ratio was significantly biased in seven of the eight populations, with males outnumbering females 3.91:1 in the lowest-altitude population. Our results support the notion of spatial segregation of sexes along on altitudinal gradient: the frequency of males decreased with altitude, from 79.6% to 41.0%. The sex ratio was not significantly correlated with population density. We also examined intersexual differences of plant height in two populations from the extremes of the studied altitudinal range. Males were the larger sex in both populations, though the difference was not significant in the high-altitude population.

Key words: Euphorbiaceae, *Mercurialis perennis*, altitudinal variation, spatial segregation, dioecy, sexual size dimorphism, sex ratio, plant height

UDC 582.682.1 : 581.5 : 575

INTRODUCTION

Ever since F i s h e r (1930), one of the most intriguing problems for evolutionary biologists has been the evolution of sex ratios (H a r d y, 2002; L e t u r q u e and R o u s s e t, 2003; O l s o n et al., 2005). In this context, spatio-temporal variation of the sex ratio and gender expression in sexually dimorphic (dioecious, androdioecious, gynodioecious) plant species, as well as spatial segregation and reproductive success of the two sex functions, have attracted much research interest (e.g., P a n n e l l, 1997; O b e s o et al., 1998; D e c k e r and P i l s o n, 2000; O r t i z et al., 2002; M o r e l l a t o, 2004). Analyzing such variation in natural populations, at both the intra- and the interpopulation level, is crucial to an understanding of pressures involved in the evolution of patterns of gender expression and the sex ratio (T r a v e s e t, 1999; O l s o n et al., 2006).

A biased sex ratio in natural populations of angiosperms has been documented in numerous studies (e.g. O b e s o et al., 1998; D e c k e r and P i l s o n, 2000; O r t i z et al., 2002). The sex ratio commonly varies in plant species with separate gender morphs (A s h m a n and D i e f e n d e r f e r, 2001) and many dioecious plants show spatial segregation of sexes along a gradient of habitat quality; such segregation has been reported mostly for wind-pollinated species (O r t i z et al., 2002).

With respect to the direction of this bias, previous studies reported a higher proportion of males at the extremes of a species range (H o f f m a n and A l l i e n d e, 1984), in resource-limited or less favorable environments (reviewed in F r e e m a n and V i t a l e, 1985), while a higher proportion of females might be expected in more favorable environments (M e a g h e r, 1988; P a n n e l l, 1997). O r t i z et al. (1998) found that male *Juniperus oxycedrus* plants were more abundant in drier places. In a number of studies, the sex ratio was reported to vary along a latitudinal (M a r i o n and H o u l e, 1996) or altitudinal (O r t i z et al., 2002) gradient.

In *Mercurialis perennis*, differences in the spatial distribution of sexes were found, correlated with light or soil conditions (C o x, 1981; W a d e et al., 1981) – for example, W a d e et al. (1981) found that in beech popu-

lations male plants were more frequent than female plants in canopy openings compared to the treebase.

Male frequency correlated positively with density in populations of *Mercurialis annua* (P a n n e l l, 1997), in accordance with the expectation that the female function is better represented when conditions are more favorable (in this case – at low densities).

Sexual size dimorphism in plants is another important issue (O b e s o, 2002). In dioecious species, the resource investment in reproduction is typically greater for females than for males, and this may result in sex-specific consequences – reduced growth or even higher mortality (W h e e l w r i g h t and L o g a n, 2004). In this context, plant height is studied as an important sexually dimorphic trait (e.g., M e a g h e r and A n t o n o v i c s, 1982; G e h r i n g and L i n h a r t, 1993; W h e e l w r i g h t and L o g a n, 2004).

The aim of this study was to examine variation of the sex ratio at different altitudes and spatial segregation between the sexes in populations of *Mercurialis perennis*, a widely distributed dioecious perennial. While its congener *M. annua* has been more studied in this respect (e.g. P a n n e l l, 1997), data for *M. perennis* are still insufficient. In addition, we examined variation in height of male and female plants in two populations from extremes of the studied altitudinal range, i.e., the lowest-altitude (196 m a.s.l.) and highest-altitude (1480 m a.s.l.) populations.

MATERIAL AND METHODS

Mercurialis perennis L. (Euphorbiaceae) is a dioecious anemophilous species with wide geographical and altitudinal distribution in Europe (G o v a e r t s, 2007). In Serbia it occupies both lowland and mountainous habitats (J a n k o v i ć and N i k o l i ć, 1972). The sexual systems expressed in the genus *Mercurialis* have attracted much research interest recently (e.g., P a n n e l l, 1997; K r ä h e n b ü h l et al., 2002).

We studied eight populations from Serbia, representing an altitudinal range of from 196 to 1480 m above sea level (site description parameters are given in Table 1); the study was carried out in the year 2006. Sex was determined from the presence of female or male flowers. Plant height was measured from ground level to the highest point on the plant (P a q u i n and A a r s s e n, 2004).

Deviations from unity for the sex ratio were verified using the chi-square test with significance adjusted by the sequential Bonferroni correction. Intersexual differences in plant height were tested using the t-test. Variables were transformed (log or arcsine) where necessary. Regression analysis was employed to examine the relationship between the sex ratio and altitude, as well as between the sex ratio and population density. All statistical analyses

Table 1. Site parameters, population density (ind/m²), and sex ratio for studied populations of *Mercurialis perennis*. The sex ratio is expressed as the proportion of males; values marked with asterisks represent significant deviations from a unity sex ratio ($\chi 2$ test with Bonferroni sequential correction, * p <0.05, ** p<0.01, *** p<0.001).

Site	Altitude	Coordinates	Aspect	Population density	Sex ratio
1. Košutnjak	196 m	44°45'30"N - 20°26'14"E	N	88.15	0.796***
2. Petnica	220 m	44°14'41"N - 19°55'59"E	N	3.82	0.789***
3. Avala	360 m	44°41'30"N - 20°30'30"E	W	27.15	0.773***
4. Ovčar	400 m	43°53'44"N - 20°10'51"E	N	2.02	0.725**
5. Povlen 1	1260 m	44°08'08"N - 19°44'33"E	NNW	19.06	0.715***
6. Povlen 2	1300 m	44°08'32"N - 19°43'36"E	W	60.25	0.669***
7. Kopaonik 1	1360 m	43°18'41"N - 20°51'06"E	E	8.65	0.532
8. Kopaonik 2	1480 m	43°18'43"N - 20°50'58"E	E	33.46	0.410*

were performed with the Statistica statistical package (v. 5.1).

RESULTS AND DISCUSSION

In the analyzed populations the sex ratio was biased, i.e., significantly different from 1:1, with males outnumbering females 3.91:1 in the lowest altitude population (Košutnjak). Deviations from unity for the sex ratio were significant in all analyzed populations except Kopaonik 1 (Table 1); in five of eight populations, this bias was highly significant ($p<0.001$).

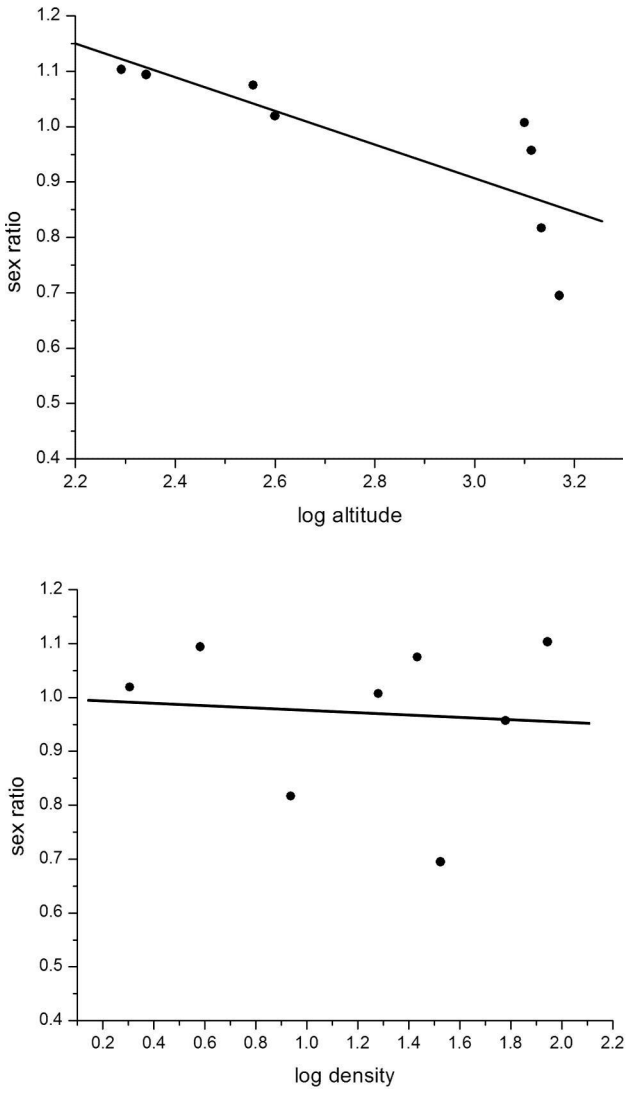

Fig. 1. Linear regression of sex ratio on a) altitude and b) population density (the sex ratio is expressed as the proportion of males).

The direction of bias changed with altitude: the frequency of males decreased from 79.6% in the lowest-altitude population to 41.0% in the highest-altitude population. Linear regression of the proportion of males on altitude confirmed a significant negative relationship (Fig. 1a, $R = -0.79$, $p = 0.018$).

However, the proportion of males showed no significant relationship with density (Fig. 1b, $R = -0.09$, $p = 0.84$).

Variation in height of male and female plants in the lowest and the highest altitude populations is shown in Table 2 and Fig. 2. At the intrapopulation level, male plants were higher than female plants in both populations, but the difference was significant only in Košutnjak population ($t = 5.68$, $p<0.001$; for population Kopaonik 2: $t = 1.73$, $p = 0.09$).

In conclusion**,** our study reveals significant variation of the sex ratio among populations of *Mercurialis perennis* and supports the notion of spatial segregation of sexes along an altitudinal gradient.

Our results are in accordance with previous findings that populations of plant species with separate gender morphs often exhibit biased sex ratios (N i c o t r a, 1998; A s h m a n and D i e f e n d e r f e r, 2001). Environmental factors have been shown to influence the proportion of sexes in several plant species (e.g., L l o y d and B a w a, 1984; M e a g h e r, 1988). Segregation by gender along the gradient of habitat quality has been attributed to a greater burden of costs associated with the female function (K o h n, 1989; S a k a i and W e l l e r, 1991).

Thus, based on previous studies, male-biased sex ratios would be expected in resource-limited, less favorable conditions, e.g., at higher altitudes. In the study of O r t i z et al. (2002) on *Juniperus communis*, the sex ratio changed along an altitudinal gradient, becoming significantly male-biased at high altitudes.

Counter to this pattern, the frequency of males in our study was highest at low altitudes and decreased with elevation, the sex ratio becoming significantly female-biased in the highest-altitude population. This pattern does not fit the expectations; however, before firm conclusions can be reached, additional information is needed. Hence, further investigations in our populations are necessary: first, to thoroughly explore determinants of habitat quality and evaluate what could be considered as "higher quality" or "lower quality"; second, to examine a number

Table 2. Descriptive statistics for plant height in the lowest- and highest-altitude populations, Košutnjak and Kopaonik 2 (height in mm, SD – standard deviation, CV – coefficient of variation, M – male plants, F – female plants, *n* – sample size).

	n	mean ± SD	median	range	CV (%)
KOŠUTNJAK					
M	35	259.5 ± 29.31	261	195-315	11.29
F	35	218.5 ± 31.13	224	144-278	14.25
KOPAONIK 2					
M	30	206.3 ± 12.01	207	179-228	5.82
F	30	200.3 ± 14.58	201.5	170-221	7.28

of additional populations from the middle part of altitudinal range (to achieve "higher resolution" with respect to the altitudinal gradient) and, particularly, from the upper extreme. In the study of O r t i z et al. (2002), for example, the sex ratio become significantly male-biased above 2600 m a.s.l.

Spatial segregation by gender consistent with the pattern previously reported for *Mercurialis perennis* in W a d e et al. (1981) was found in only one of the eight analyzed populations (in the lowest-altitude population – Košutnjak). In all other populations, it was absent; thus, we cannot consider it a general pattern in this species.

In addition, correlations of male frequency with density in *M. perennis* were not significant, contrary to findings in *M. annua* (P a n n e l l, 1997). Though the current hypothesis predicts that females of dioecious species are more adversely affected than males when resources are limiting under stressful conditions, some studies showed the opposite – e.g., in the study of G e h r i n g and L i n h a r t (1993) males and females did not respond differently to low resource availability.

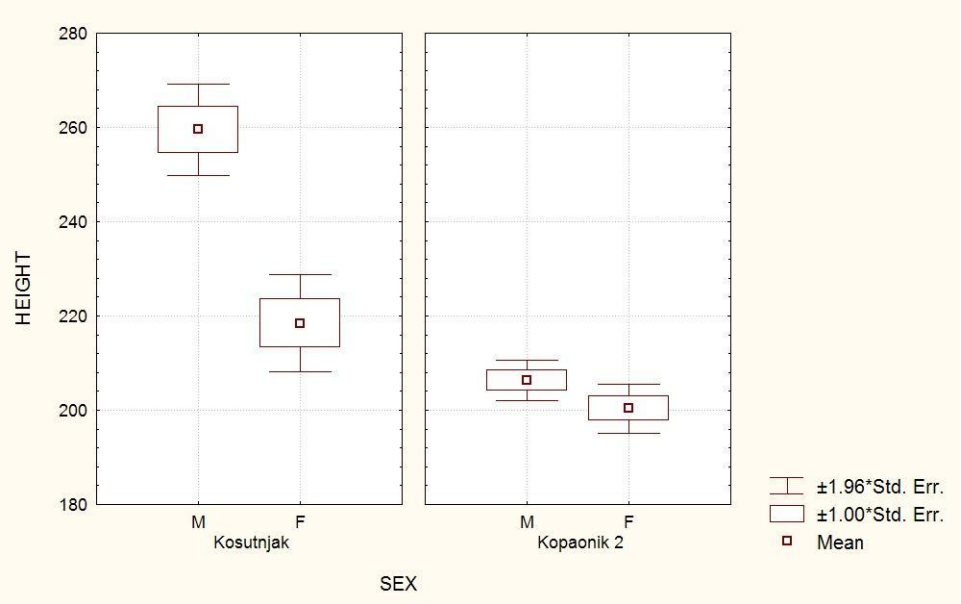

Fig. 2. Intersexual variation in plant height in the lowest- and highest-altitude populations, Košutnjak and Kopaonik 2 (height in mm, means ± standard errors; M – male plants, F – female plants).

Plant height is often studied as a sexually dimorphic trait (G e h r i n g and L i n h a r t, 1993). Dioecious plants are suitable for assessing variation in plant size and intersexual differences in resource investment. Females of dioecious plant species typically invest more in reproduction than males (though there are exceptions – e.g., D e l p h et al., 2005) and this may affect the growth rate, making it lower in females (W h e e l w r i g h t and L o g a n, 2004). The overview in O b e s o (2002) on differences in reproductive investment as a determinant of sexual dimorphism shows that females of woody dioecious species are consistently the smaller sex, while females of herbaceous perennials are often the larger sex – which contradicts expectations of the hypothesis.

In our study, however, the preliminary results on intersexual differences in plant height generally fit the hypothesis – male plants were larger in both populations (though the difference was not significant in the high-altitude population). As expected, average height and intersexual differences were smaller in the high-altitude population. In the study on *Juniperus communis* (O r t i z et al., 2002) growth decreased with increasing altitude, but was similar in both sexes.

Thus, our results contribute to a growing but contradictory body of data on sex ratio variation and intersexual differences in plants; further investigation is needed to resolve and fully understand these problems.

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ВИСИНСКО ВАРИРАЊЕ ОДНОСА ПОЛОВА И ПРОСТОРНА СЕГРЕГАЦИЈА КОД ДВОДОМЕ ВРСТЕ *MERCURIALIS PERENNIS* L. (EUPHORBIACEAE) У СРБИЈИ

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Један од најзанимљивијих проблема за еволуционе биологе представља однос полова. Просторно-временско варирање учесталости мушких јединки код сексуално диморфних врста биљака, просторна сегрегација и различито учешће мушког и женског пола предмет су интензивних истраживања. У овом раду истраживали смо висинско варирање односа полова, као и просторну сегрегацију полова код врсте *Mercurialis perennis* L., дводоме анемофилне врсте широког ареала. Испитали смо осам популација у опсегу надморских висина између 196 м и 1480 м. Однос полова је значајно одступао од 1:1 у седам од осам популација, са односом 3,91:1 у корист мушких јединки у популацији на најнижој надморској висини. Резултати подржавају претходна запажања о просторној сегрегацији полова дуж висинског градијента: учесталост мушких јединки се смањивала са порастом надморске висине - од 79,6% до 41,0%. Однос полова није био значајно корелисан са густином популације. Истражили смо и разлике у висини мушких и женских биљака у популацијама са најмање и највеће надморске висине. У обе популације мушке биљке су биле више, мада разлика није била статистички значајна у високопланинској популацији.