

Sex ratio and spatial distribution of male and female *Antennaria dioica* (Asteraceae) plants

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Abstract

Sex ratio, sex spatial distribution and sexual dimorphism in reproduction and arbuscular mycorrhizal colonisation were investigated in the dioecious clonal plant *Antennaria dioica* (Asteraceae). Plants were monitored for five consecutive years in six study plots in Oulanka, northern Finland. Sex ratio, spatial distribution of sexes, flowering frequency, number of floral shoots and the number and weight of inflorescences were recorded. In addition, intensity of mycorrhizal fungi in the roots was assessed. Both sexes flowered each year with a similar frequency, but the overall genet sex ratio was strongly female-biased. The bivariate Ripley's analysis of the sex distribution showed that within most plots sexes were randomly distributed except for one plot. Sexual dimorphism was expressed as larger floral and inflorescence production and heavier inflorescences in males. In addition, the roots of both sexes were colonised to a similar extent by arbuscular mycorrhizal fungi. The female sex-biased flowering ratios reported are not consistent among years and cannot be explained in terms of spatial segregation of the sexes or sex lability. The possible reasons for the female-biased sex ratio are discussed.

Key words: sexual spatial patterns, dioecy, sex ratio, Ripley's analysis, sexual dimorphism.

1. Introduction

The occurrence of dioecy is reported in about 6% of all angiosperm species (Renner and Ricklefs 1995). Dioecy is believed to have arisen due to the promotion of outcrossing in cosexuals (Stebbins 1957; Baker 1984) and/or as a result of selection of different reproductive optima for each sex (Charnov 1982; Charlesworth and Morgan 1991). Resources needed for reproduction differ between male and female plants since reproductive investment in males is limited to flowering, whereas females need not only to produce flowers, but also seeds which are more expensive to produce. Therefore, costs of reproduction are usually higher for female plants compared to male plants and consequently, female plants usually invest relatively more resources to reproduction and less in maintenance and growth than males (reviewed in Obeso 2002). These sex-differential patterns of resource allocation may cause sex-specific life histories, as all plant activities compete for the same limited resources (Cody 1966; Charnov 1982). Thus, it is not surprising to detect that sex-differential resource allocation patterns result in different growth rates (e.g. Oyama 1990; Obeso 1997) or different mortality patterns between the sexes (e.g. Ågren 1987; Cipollini et al. 1994), resulting in the often observed biased sex ratios in dioecious plant populations (e.g. Armstrong and Irvine 1989; Thomas and LaFrankie 1993). However, biased sex ratios may also result from sexual spatial segregation (SSS) if the sexes occupy different parts of the same habitat. Many studies report SSS in dioecious species along environmental gradients. These gradients include water (Dawson and Ehleringer 1993), altitude (Engelskjøn and Skifte 1995), light (Barrett and Thomson 1982), nutrients (Eppley 2005), pH (Cox 1981), and soil salinity (Freeman et al. 1976). The general trend arising from these observations is that female plants are usually found in less harsh parts of the environment compared to males, which has been associated with their higher costs of reproduction (Freeman and McArthur 1982). The mechanisms leading to SSS include sex-associated differential germination (Eppley 2001), differential mortality of males and females in different sites (e.g. Meagher and Antonovics 1982; Gibson and Menges 1994), the ability to switch

sex based on the environmental conditions (reviewed in Korpelainen 1998), and the different use of resources by the two sexes (Dawson and Geber 1999; Obeso 2002).

Sex-differential resource allocation patterns are also associated with sexual dimorphism (differences between individuals of different gender in traits other than sex itself, reviewed in Geber et al. 1999), a widespread phenomenon in plants. Sexual dimorphism results from the sex-specific patterns of resource allocation associated to the costs of the sexual functions and how the sexes achieve their fitness (reviewed in Case and Ashman 2005). In dioecious plants, females generally experience greater costs of reproduction than males (reviewed in Obeso 2002). This may result in males achieving reproductive maturity earlier than females (e.g. Krischik and Denno 1990; Yamashita and Abe 2002) and in polycarpic plants, males reproducing more frequently than females (reviewed in Delph 1999). Intensive flowering and seed production may deplete resources and thus prevent flowering in the following year or years (Reekie and Bazzaz 2005). Moreover, flowering frequency is affected by the environmental conditions. Although a critical parameter defining plant fitness, flowering frequency is rarely measured since monitoring the same individual over several years is necessary.

A wide variety of host plants form symbiosis with arbuscular mycorrhizal (AM) fungi in their roots (Wang and Qiu 2006). In AM symbiosis, nutrients and water are delivered to the plant from the soil whereas the fungi receive carbon in exchange (Smith and Read 1997). It has recently been shown that the sexes of dioecious (Varga and Kytöviita 2008; Varga and Kytöviita 2010a) and gynodioecious plants (Varga and Kytöviita 2010b) may gain sex-specific benefits from their AM symbionts and different patterns of root colonisation have been reported between the sexes of dioecious plants (Eppley et al. 2009). This sexual dimorphism between plants and AM fungi has been related to the different resource needs and allocation patterns between the genders (reviewed in Varga 2010). Recently, sexual dimorphism in biotic interactions with AM fungi is receiving increasing attention (Gehring and Whitham 1992; Varga and Kytöviita 2008; Eppley et al. 2009;

Varga et al. 2009; Vega-Frutis and Guevara 2009; Botham et al. 2009; Varga and Kytöviita 2010a,b,c).

Regardless of the huge volume of work dealing with dioecious perennial plants, surprisingly few studies have followed the same individuals for several flowering seasons. In addition, sexual dimorphism in symbiotic relationships is rarely reported. To elucidate these caveats, we monitored *Antennaria dioica* in the field for five consecutive years. The specific aims of the present study were to investigate any sex ratio bias within surveyed plots; to determine sex spatial distribution within these plots; and to investigate possible differences between the sexes in reproduction frequency and AM colonisation. According to the theory that females have larger costs of reproduction than males, we predicted that (1) *A. dioica* sex ratio would be male-biased if the costs of reproduction are indeed larger for females compared to males; alternatively, (2) females and males would be spatially segregated within the area, or (3) if SSS does not occur, males would flower more often and will produce more flowers than females.

2. Materials and methods

2.1 Plant species

Antennaria dioica (L.) Gaertn. (Asteraceae) is a dioecious, perennial clonal plant that grows in heaths, dry grasslands, and sandy or stony places. In addition, *A. dioica* is sometimes found in semiopen forests. It is widely distributed in temperate regions of the northern hemisphere (Tutin et al. 1976) even though populations have been reported to be declining in Fennoscandia (Von Numers and Korvenpää 2007) probably due to the loss of suitable habitats and its relatively poor colonising ability (Eriksson 1997). Each genet may produce one to several ramets by clonal growth of surface creeping stolons. Genets flower producing up to one flowering shoot per ramet. In Finland, flowering takes place between June and July and the species is pollinated by generalist

insects from several orders including Lepidoptera, Hymenoptera, Diptera, and Coleoptera (Willis and Burkill 1903).

2.2 Study plots

Genets were monitored for five consecutive years (2005 to 2009) in Oulanka, northern Finland. In 2005, we randomly marked four plots (A1 – A4; Table 1) where *A. dioica* was the dominating plant species. Unfortunately, A2 was lost to human activities in 2006 and thus, this plot was excluded from further analyses. In exchange, two new study plots were included in 2006 (A5 – A6; Table 1). The plants were growing in disturbed soil at road verge under open sky. The closest neighbouring species to *A. dioica* were *Vaccinium myrtillus*, *V. vitis-idaea*, *Calluna vulgaris* and *Cladonia* lichens. Soil pH and organic matter content of the study plots are given in Table 1. Mean precipitation and temperature in Oulanka during the course of the observations is given in Fig. 1.

2.3 Plant measurements

In 2005, all flowering genets within each plot were sexed and permanently marked. In the following years, all new flowering individuals within each plot were also sexed and marked. Each flowering season, the number of floral shoots and the number of inflorescences produced per genet were recorded. Because floral shoots and inflorescences remain long time after seed dispersal, each study plot was checked only once when most of the genets were already fruiting. Estimations of the number of genets were possible as the different genets did not generally overlap in the study plots. Differences in colour and leaf's shape helped also in identifying the genets. Nevertheless, results should be interpreted with caution since the number of genets may be overestimated and molecular analyses should be employed to verify genet identity.

In 2007, inflorescences from 17 male and 17 female genets were randomly selected from a plot not included in the surveys to calculate inflorescence mass after drying the flowers at 80°C for 8 hours.

We also selected another 20 male and 20 female genets to assess arbuscular mycorrhizal status of the different sexes. Root samples from each genet were taken using a 2 cm core in diameter. Roots were cleaned under running water to remove adhered soil particles, and stored in 50% ethanol. Roots were later stained after clearing by incubation in 10% KOH for four days at room temperature and additional incubation in 1.5% H₂O₂ for two hours at room temperature. After two hours in 1% HCl, roots were incubated at 80 °C in 0.02% trypan blue staining solution for two hours. Using a microscope at 100 x magnification, the AM fungal colonisation was measured as the proportion of intersects with hyphae, vesicles and arbuscules in 10 root segments of two cm length. One hundred intersects per sample were observed.

2.4 Soil measurements

Five samples per site were randomly taken using 5 cm diameter core to determine soil pH, and soil organic matter content (Table 1). Soil samples were passed through 2 mm mesh sieves and kept at 4°C until analyses. Soil pH_{H2O} was determined using the method as described in van Reeuwijk (1986): 25 ml of fresh weight substrate was shaken for two hours at 200 rotations min⁻¹ in 62.5 ml distilled water. After 30 minutes sedimentation, pH was determined. Organic matter content was calculated after burning the sample at 480°C for 5 hours.

3. Data analyses

3.1 Sex ratios

Flowering sex ratio and population sex ratio were calculated for each plot and year. Flowering sex ratios are expressed as the proportion of flowering males divided by all flowering genets [flowering

males/(flowering males + flowering females)], rather than a strict sex ratio, because analysis of ratios *sense stricto* can lead to errors in interpretation (Wilson and Hardy 2002). Population sex ratio is expressed as number of male genets / (female + male genets), excluding sex labile genets. Deviations of the flowering and the population sex ratio from 1:1 were tested using binomial tests.

3.2 Spatial analyses

The occurrence of spatial segregation was investigated using Ripley's K analysis (1981). In order to remove scale-dependency and stabilise the variance, the L function (square root transformation of K/π) is used instead of the K function (for calculation of K and L functions and the significance of these functions see Wiegand and Moloney 2004 and Perry et al. 2006). Each population was first analysed using Ripley's univariate analysis to examine whether the population as a whole was randomly distributed in space. Therefore, all individuals regardless of the gender were included and tested against the null hypothesis of complete spatial randomness using 1000 Monte Carlo simulations. Ripley's bivariate analysis was later used to examine spatial association between female and male genets (excluding sex labile genets) using the null model of random labeling of sexes to existing positions of plants using 1000 Monte Carlo simulations. Analyses were conducted with the statistical package Spatstat from R (Baddeley and Turner 2005). Ripley's isotropic edge correction was used to account for edge effects in the analyses. In the figures, $L(r) - r$ and the 95% confidence envelopes are given. Any distance at which the $L(r) - r$ statistic falls outside the confidence envelopes indicate significant departure from a random distribution: $L(r) - r$ above the upper confidence interval denotes clumping, and $L(r) - r$ below the lower envelope denotes over-dispersal at any distance given. Values of $L(r) - r$ within the confidence envelope indicate random distribution of points.

3.3 Plant parameters

Differences in plant mortality and sex lability were investigated with binary logistic regression models. Number of floral shoots and inflorescences produced were analysed separately for each year to examine sexual dimorphism in reproduction across the years. In addition, differences in number of floral shoots and inflorescences between sexes and populations were tested using the total accumulated number of floral shoots and inflorescences produced during the five years. Data were not normally distributed and therefore were analysed using a generalised linear model with a Gamma distribution and identity link function. The proportion of root length colonised by hyphae, arbuscules and vesicles was arc sin square root transformed prior analyses. These data were analysed with ANOVA models with plant sex (M, F) as fixed factor and population as a random factor. Spearman's rank correlation analyses were employed to assess the relationship between the observed flowering sex ratio of any given year with the monthly precipitation and temperature from January till June of the same year. All statistical analyses were conducted with SPSS v.16.0 (SPSS, Chicago, Illinois, USA).

4. Results

4.1 Mortality and sex lability

Plant mortality and sex lability were very low and only two plants out of the 274 monitored died in the study plots. Sex lability was found in three out of five plots monitored (excluding A2 as it was monitored just once). Altogether nine individuals showed sex lability but there was no consistent pattern: three genets changed from male to female, two genets changed from female to male, and the remaining four genets changed twice (male-female-male or female-male-female) during the study period.

4.2 Flowering and sex ratios

A total of 274 genets flowered at least once during the five years. The proportion of flowering genets varied among plots and years (Table 2) with a minimum reached in 2007 (only 15% of the genets flowered) and a maximum in 2005 (56% of the genets flowered). The overall genet sex ratio was strongly female-biased; although varying among plots and years it was never male-biased (Table 2). In 2005 and 2006, plots showed female-biased flowering sex ratio which was not detected in the following three years (Table 2). The observed flowering sex ratio of any given year was only positively correlated with monthly precipitation and average temperature in February as shown by the Spearman's rank correlation analyses (Spearman rho = 0.900, $P = 0.04$, $df = 5$, $R^2 = 0.77$; other correlations were $P > 0.19$). This indicates that the warmer the average temperature in February, the more males would flower, since the correlation was only significant for the number of males flowering ($P = 0.04$), not for females ($P = 0.104$).

The frequency of reproduction (i.e. number of years when flowering/ number of years monitored) in individuals monitored more than once (thus excluding A2) was similar between female and male genets (1.6 years out of 5 in females vs. 1.9 in males, Mann-Whitney $U = 5148$, $P = 0.67$). A small proportion of individuals flowered during two consecutive years and this proportion was similar between sexes across the years (2006: 6 vs. 12%; 2007: 1 vs. 5%; 2008: 5 vs. 8%; 2009: 14 vs. 19% for females vs. males respectively).

4.3 Spatial distribution

Visually, it seems that within each study plot genets are randomly distributed in space (Fig. 2). This is confirmed by the univariate Ripley's analysis which shows that individuals are statistically randomly distributed in most plots. The $L(r) - r$ statistic falls between the two confidence interval for most of the distances considered except for A2 and A4 where the genets are clustered at distances larger than 35 and 20 cm respectively (Fig. 3).

The bivariate Ripley's analysis of the distribution of one sex regarding the other provided no significant indication of spatial segregation or aggregation in all plots (Fig. 4), suggesting that females and males grow randomly with respect to each other at any scale (Fig. 4).

4.4 Mycorrhizal status of the plants

The roots of both male and female *A. dioica* were intensively colonised by AM fungi and the sexes did not differ in the proportion of root length colonised by the different fungal structures considered (Hyphae: 43.9 ± 5.9 vs. 42.5 ± 7.8 for females and males respectively, $F_{1,35} = 0.022$, $P = 0.88$; vesicles: 8.5 ± 1.9 vs. 12.3 ± 3.7 for females and males respectively, $F_{1,35} = 0.908$, $P = 0.35$; arbuscules: 12.9 ± 2.5 vs. 9.4 ± 3.1 for females and males respectively, $F_{1,35} = 1.440$, $P = 0.24$).

4.5 Reproductive parameters

There was variation in the number of floral shoots and inflorescences produced between years (Table 3). Within the study years, males produced more floral shoots than females in three out of five years they were monitored, even though this difference was only statistically significant in some populations as indicated by the interaction between gender and population (Table 3).

Similarly, the sexes did not differ in the number of inflorescences per floral shoot (5.40 ± 0.11 vs. 5.46 ± 0.18 for females and males respectively, range = 1-12, $X^2_1 = 0.010$, $P = 0.92$), again with some variation among populations ($X^2_5 = 100.552$, $P < 0.01$). Therefore, the number of inflorescences per genet was larger in males plants compared to females in three years, again with variation among populations (Table 3). Taken together, the accumulated total shoots and inflorescences production during the five years was 2.1 and 2.3 times larger in males compared to females depending on the population. In addition, males produced statistically significantly heavier inflorescences than females ($2.47 \pm 0.06 \mu\text{g}$ vs. $1.33 \pm 0.03 \mu\text{g}$ in males and females, respectively, $F_{1,16} = 73.53$, $P < 0.01$).

5. Discussion

Generally, females are considered to invest relatively more into reproduction than males (Obeso 2002). However, in *Antennaria dioica*, females do not seem to possess larger costs of reproduction than males. Sexual dimorphism in this species was manifested as larger shoot and inflorescence production and heavier inflorescences in males, with both sexes showing equal flowering frequency. Even though we did not measure the investment in seed production, it seems unlikely that resources allocated to this function could result in a difference in reproductive costs between the sexes since *A. dioica* produces relatively small seeds. Therefore, it seems prudent to assume that in *A. dioica* the cost of reproduction may be similar between the sexes. Also other researchers report equal cost of reproduction in some plant species (see references in Delph 1999 and Obeso 2002). The work of Lloyd and Webb (1977) and Bell (1980) predicts that when the two sexes invest similarly in reproduction, no differences in life-history traits should occur. Therefore, the similar costs of reproduction found between the two sexes in our study species can explain the lack of differences in reproduction frequency observed among the five study years. Concurring with that, we observed a similar proportion of root length colonised by AM fungi in both male and females agreeing with most studies reporting similar root colonisation by AM fungi in sexually dimorphic plants (Gehring and Whitham 1992; Varga & Kytöviita 2008; Varga et al. 2009; Varga and Kytöviita 2010b). Species belonging to the AM fungal genera *Glomus* and *Acaulospora* have been identified colonising the plants in the field (Santos-González et al. 2007). Lack of sexual difference in the amount of symbiotic fungal structures in plant roots suggests that both sexes have similar resource expenditure on symbiotic functions.

In plant populations, departs from 1:1 sex ratio have been frequently reported. Recently, Barrett et al. (2010) reviewed the population sex ratios of 126 dioecious species and showed that biased sex ratios are common in plants, with male bias (46% of species) being over twice as

frequent as female bias (21% of species). Our estimated sex ratio from the Finnish populations agrees well with previous *A. dioica* female-biased flowering sex ratios reports (Eriksson 1996; Öster and Eriksson 2007). Even though female-biased sex ratios are less commonly found than the male-biased, several mutually not exclusive factors have been proposed to explain this result in biased sex ratio. The bias direction will depend on which gender is favoured by the mechanism; therefore the following factors can be also used to explain male-biased sex ratios: (i) a biased primary sex ratio, (ii) different germination requirements, (iii) differences in time of initial reproduction, (iv) different mortality associated with the sexes, (v) spatial segregation of the sexes, and (vi) sex lability.

A biased sex ratio already present in the seeds (i) has been reported for several species (Webb 1992; Taylor 1996; Wolf et al. 2001; de Jong and van der Meijden 2004), even though in species with genetic sex determination a 1:1 seed sex ratio is predicted (Fisher 1930). In addition, given an unbiased primary sex ratio, both genetic and environmental factors can potentially modify the sex ratio of seeds (see Stehlink et al. 2008; and references therein). For example, Stehlink et al. (2008) demonstrated that in four of six populations studied, female *Rumex nivalis* positioned in close proximity to males captured more pollen and exhibited more female-biased sex ratios, probably by selective fertilisation resulting from pollen tube competition. Since the primary sex ratio in *A. dioica* is currently unknown this factor can not be ruled out as one factor responsible for the female-biased sex ratio observed.

Differences in germination requirements of the sexes (ii) could also contribute to biased adult sex ratios. Relatively few studies have documented emergence time differences between the sexes. Nevertheless, these differences may have important consequences on later survival and reproduction (see Purrington and Schmitt 1998; and references therein). The importance of sexual differences in germination needs is currently unknown for *A. dioica*. Differences due to the time needed to reach initial reproduction (iii) could also contribute to female-biased sex ratios if females

start reproducing earlier than males. In the majority of species studied males mature earlier than females in relation to their smaller investment into reproduction (Delph 1999). However, it seems logical to assume that this is not the pattern exhibited by *A. dioica* even though it remains unknown.

Another factor determining biased sex ratios is different mortality of the sexes (iv). Females are expected to have higher mortality because of their greater investment during reproduction (Obeso 2002) even though sex differential mortality has been reported also during early life stages (Eppley 2001). Under favourable conditions mortality should be minimal and sexes will be present in the ratio as presented in seeds, while under stress conditions the more vulnerable sex will suffer higher mortality. We monitored only adult (flowering) plants and the mortality was low and no differences between sexes were notable, again in agreement with the lack of differences in reproductive costs. Higher male juvenile mortality remains, however, a possible explanation for the female biased sex ratio in this species.

Sexual spatial segregation (SSS) may also create local female-biased sex ratios (v). Even though SSS in dioecious species has been repeatedly observed (e.g. Bierzychudek and Eckhart 1988), our results from the random point process analysis revealed that female and male *A. dioica* were mostly randomly distributed within the study plots. SSS can be generated in a variety of ways including sex-associated differential germination, mortality associated with flowering, and sex-change patterns (Freeman et al. 1976; Freeman and McArthur 1984; Bierzychudek and Eckhart 1988). In addition, this segregation could be a consequence of intrasexual competition. For example, Nanami et al. 2005 reported females in *Podocarpus nagi* (Podocarpaceae) were more sensitive to the presence of neighbours because of higher reproductive costs than males. Nevertheless, we could also exclude this factor as the responsible for the pattern observed in our *A. dioica* study populations as females and males were randomly distributed in space.

Individuals of a large number of dioecious species are able to change their sexual expression showing sex lability (reviewed in Freeman et al. 1980; Korpelainen 1998) and this may cause

biased sex ratios (vi). Two mutually not exclusive explanations have been proposed to explain sex lability. First, the size advantage model (Ghiselin 1969; Charnov 1982) predicts that plant change their sex expression when size- or age- specific reproductive success differs between males and females. Accordingly, sex changes with increased size (Bierzychudek 1982) or age (Lloyd and Bawa 1984) have been demonstrated. Second, several environmental conditions have been reported to be responsible for switching sex (reviewed in Korpelainen 1998). Sex changes in plants have been considered as a strategy of sex allocation to enhance fitness over the lifetime of plants (Freeman et al. 1980; Policansky 1981; Lloyd and Bawa 1984). Sexually labile plants have the potential to have either female, male or both sex organs, with some environmental feature determining which genes are switched on. In *A. dioica* sex expression has been observed to be labile (Freeman et al. 1980; this study). However, the low proportion of individuals changing their sexual expression in the present study does not allow making strong conclusions on advantage of sex lability or conditions that induce it. Given that the current study was not initially designed to investigate sex lability, further investigations are warranted in order to elucidate the importance and incidence of sex lability for *A. dioica*.

To summarise, in this study we have shown that sexual spatial segregation, differences in adult mortality, sexual dimorphism in flowering frequency, allocation to root symbionts or to reproduction does not explain female biased populations in *Antennaria dioica*. Future investigations using sex-specific molecular markers should be employed to identify whether sexual differences in juvenile mortality or primary sex ratio are responsible for the sexual bias in this species.

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Table 1. Location and soil properties of the plots (A1 – A6) included in the study across Oulanka National Park.

Code	Coordinates	Plot size (m²)	pH	OM (%)
A 1	66°21'59"N, 29°31'51"E	12	5.06 ± 0.18	3.6 ± 0.2
A 2	66°21'48"N, 29°25'27"E	10	5.99 ± 0.27	3.1 ± 1.1
A 3	66°22'42"N, 29°19'52"E	12	6.04 ± 0.29	2.3 ± 0.3
A 4	66°21'48"N, 29°25'27"E	9	6.46 ± 0.25	3.0 ± 0.1
A 5	66°21'39"N, 29°21'24"E	21	5.60 ± 0.14	3.6 ± 0.4
A 6	66°22'05"N, 29°29'05"E	15	5.97 ± 0.07	2.2 ± 0.3

Table 2. Number of individuals monitored (Ind), genet sex ratio (excluding labile plants), and flowering sex ratio (calculated from observed expressed gender) among the study years. Sex ratios are indicated as (males / males + females) followed by the number of genets in brackets.

Code	Ind	Sex ratio	2005	2006	2007	2008	2009
A 1	31	0.46 (28)	0.54 (13)	0.55 (11)	0.50 (2)	0.48 (27)	0.54 (13)
A 2	46	0.33* (46)	0.33* (46)	-	-	-	-
A 3	64	0.38† (60)	0.39 (36)	0.39 (23)	0.33 (3)	0.48 (29)	0.25† (12)
A 4	84	0.28*** (82)	0.31* (52)	0.22* (18)	0.23† (13)	0.38 (29)	0.36 (14)
A 5	22	0.32† (22)	-	0.33 (9)	0.25 (8)	0.50 (10)	0.44 (9)
A 6	27	0.30* (27)	-	0.26* (19)	0.57 (7)	0.39 (18)	0.42 (17)
All	274	0.34*** (264)	0.37*** (101)	0.34** (80)	0.33 (33)	0.44 (113)	0.40 (65)

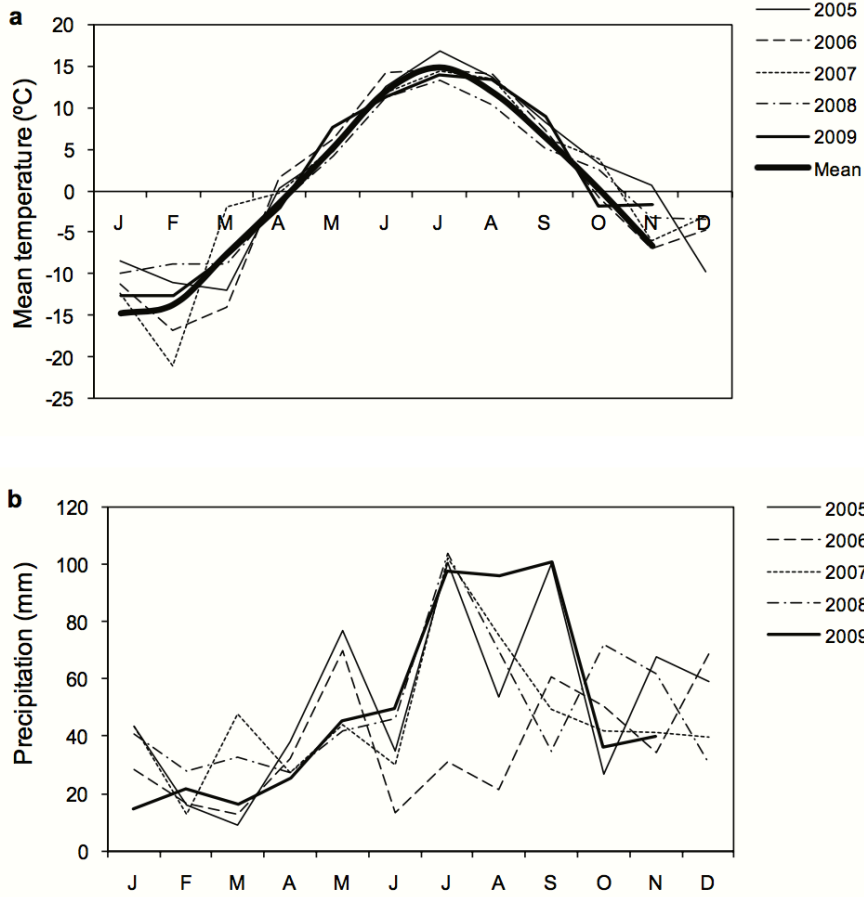
Asterisks indicate significant deviations from a 1:1 sex ratio according to binomial tests at † $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$.

1 **Table 3.** Number of floral shoots (# Shoots), and number of inflorescences per genet (# Inflo.) across the study years and total accumulated
2 production (Accumulated) in female, male and labile *Antennaria dioica* plants. Mean \pm SE are reported followed by the number of genets
3 observed in brackets. The statistical results of the Generalised linear models are also indicated by the Wald X^2 followed by its significance level.
4 Significant results are shown in boldface.

Variable	Sex	2005		2006		2007		2008		2009		Accumulated	
# Shoots	Female	2.03 \pm	0.22 (92)	1.83 \pm	0.19 (47)	2.35 \pm	0.56 (17)	1.84 \pm	0.20 (63)	2.27 \pm	0.63 (37)	2.99 \pm	0.27 (139)
	Male	2.39 \pm	0.27 (46)	4.58 \pm	1.51 (24)	1.70 \pm	0.30 (10)	3.93 \pm	1.37 (45)	3.60 \pm	0.76 (25)	6.25 \pm	1.69 (73)
	Labile	1.63 \pm	0.50 (8)	1.50 \pm	0.38 (8)	1.00 \pm	- (1)	3.67 \pm	1.41 (6)	4.67 \pm	3.18 (3)	6.20 \pm	2.30 (10)
		X^2	<i>P</i>	X^2	<i>P</i>	X^2	<i>P</i>	X^2	<i>P</i>	X^2	<i>P</i>	X^2	<i>P</i>
	Sex	5.652	0.017	7.660	0.006	1.113	0.291	9.644	0.002	2.816	0.093	13.553	<0.001
	Population	32.742	<0.001	22.237	<0.001	4.443	0.349	32.136	<0.001	22.829	<0.001	33.242	<0.001
	Sex x Population	5.867	0.118	15.131	0.004	0.615	0.961	20.894	<0.001	15.739	0.003	14.739	0.005
# Inflo.	Female	12.08 \pm	1.44 (92)	9.64 \pm	1.16 (47)	10.71 \pm	3.14 (17)	10.81 \pm	1.34 (63)	7.61 \pm	1.03 (31)	14.75 \pm	1.23 (139)
	Male	15.00 \pm	2.08 (46)	26.29 \pm	10.23 (24)	8.20 \pm	1.97 (10)	22.7 \pm	10.01 (45)	18.52 \pm	4.20 (21)	33.90 \pm	11.56 (73)
	Labile	9.25 \pm	3.38 (8)	9.25 \pm	3.00 (8)	4.00 \pm	- (1)	21.50 \pm	7.46 (6)	19.00 \pm	15.10 (3)	33.80 \pm	12.08 (10)
		X^2	<i>P</i>	X^2	<i>P</i>	X^2	<i>P</i>	X^2	<i>P</i>	X^2	<i>P</i>	X^2	<i>P</i>
	Sex	4.574	0.032	20.183	<0.001	0.002	0.963	2.908	0.088	62.585	<0.001	12.406	<0.001
	Population	36.367	<0.001	165.446	<0.001	24.523	<0.001	165.132	<0.001	149.645	<0.001	35.656	<0.001
	Sex x Population	5.681	0.128	55.278	<0.001	7.940	0.094	43.601	<0.001	63.488	<0.001	12.482	0.014

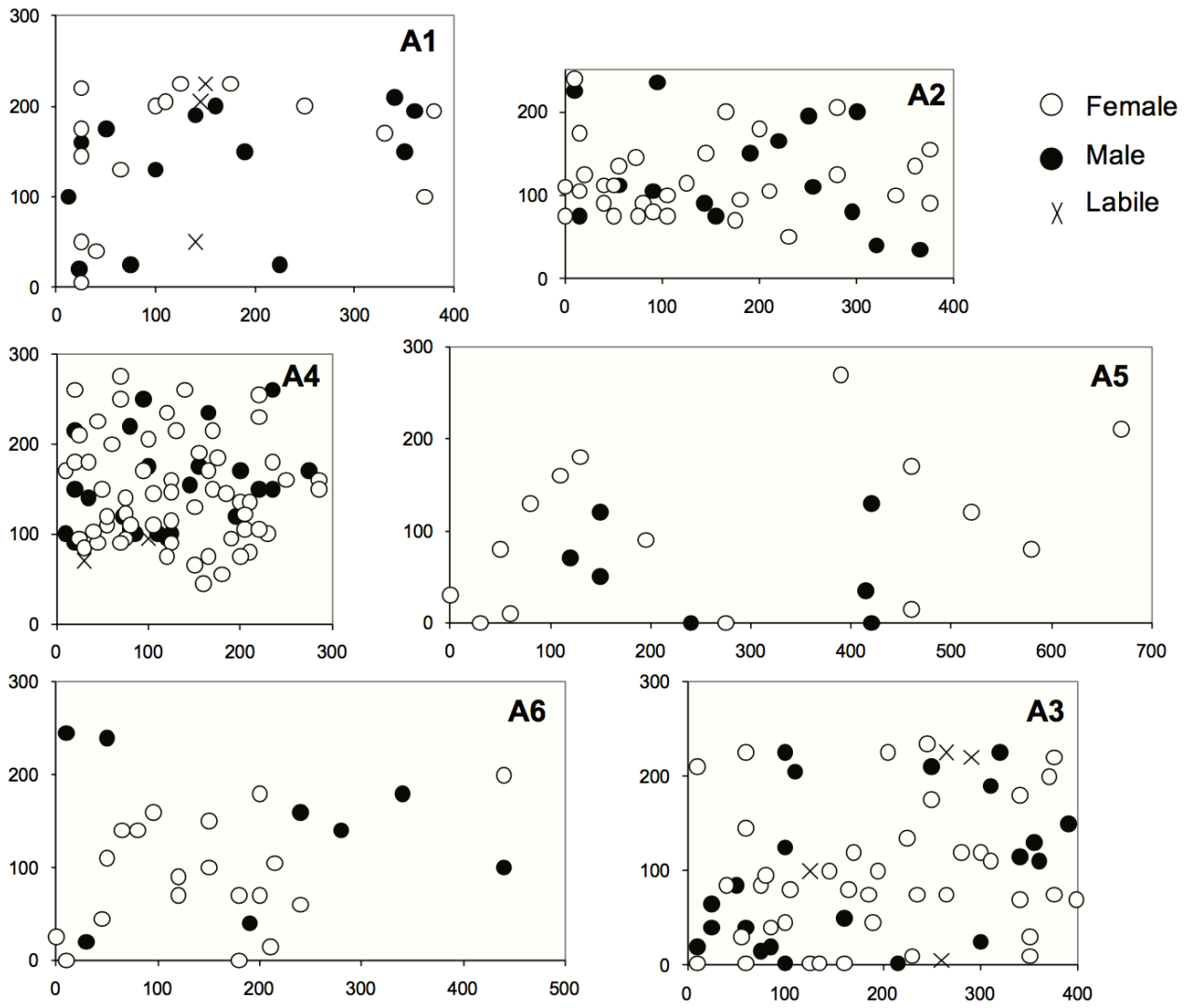
5 **Figure captions**

6 **Fig. 1.** (a) Monthly averaged mean temperature (°C) and (b) monthly accumulated precipitation
7 (mm) measured for Oulanka during the years of the study. In (a) the mean values calculated from
8 1967 – 2009 are indicated with a thicker line.



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17 **Fig. 2.** Distribution of male (solid circle), female (open circle), and labile genets (cross) in the study
18 plots. Axes are in cm.



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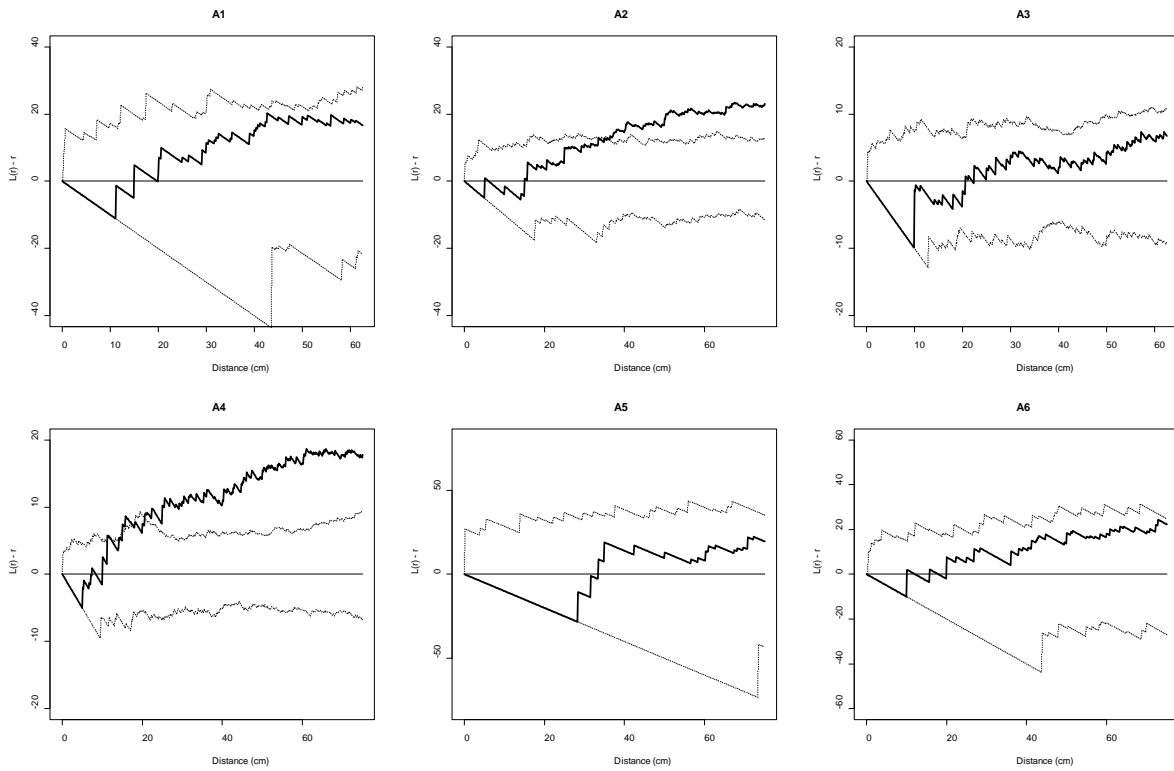
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27 **Fig. 3.** Results of Ripley univariate $L(r)$ analysis of *A. dioica* plants distribution in the study plots.
28 Ripley's L function is represented by solid lines. Dashed lines represent 95% confidence intervals
29 generated from 1000 Monte Carlo simulations of a randomly distributed population. $L(r)$ values
30 within the confidence interval indicate random association, above the interval indicate significant
31 association, and below the interval indicate significant segregation between plants.



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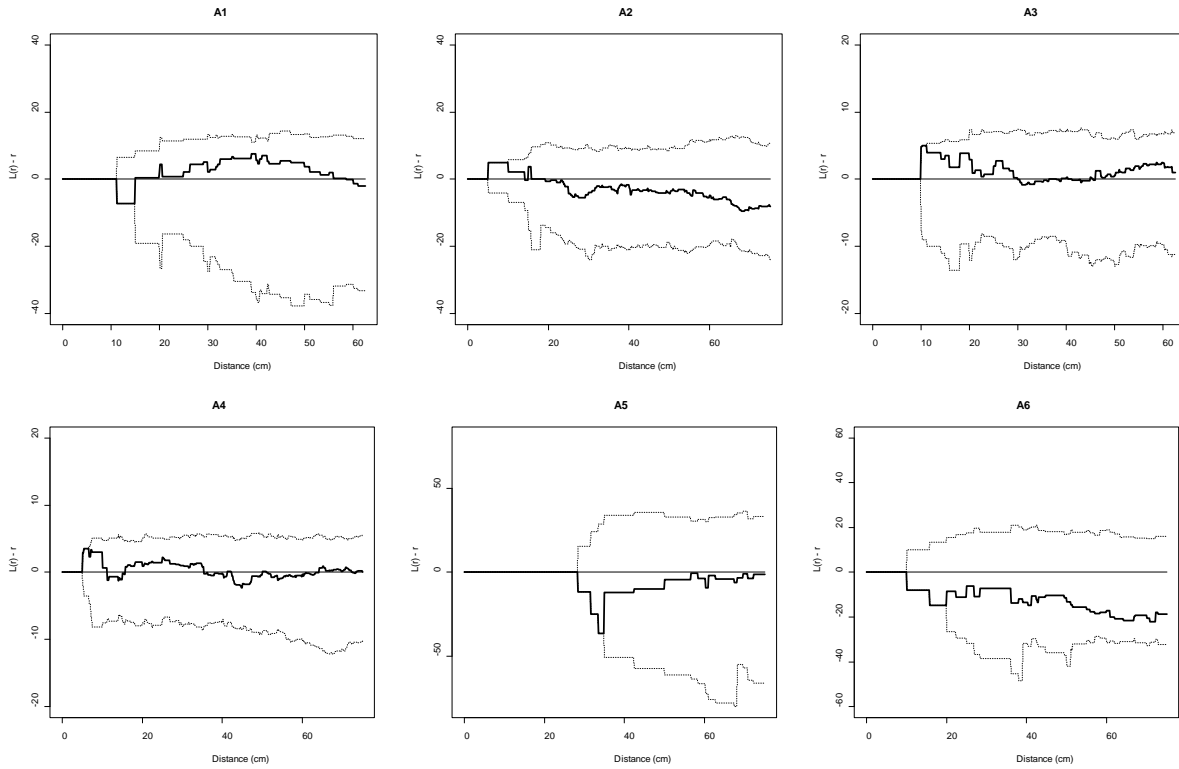
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43 **Fig. 4.** Results of Ripley bivariate $L(r)$ analysis of male and female *A. dioica* plants in the study
44 plots. $L(r)$ values are represented by solid lines. Dashed lines represent 95% confidence intervals
45 generated from 1000 Monte Carlo simulations of a randomly distributed population. $L(r)$ values
46 within the confidence interval indicate random association, above the interval indicate significant
47 association, and below the interval indicate significant segregation between males and females.



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