Generative reproduction dynamics in populations of the perennial herb *Polygonatum multiflorum* (Asparagaceae)

Igor Kosiński

Department of Biology and Pharmaceutical Botany, Medical University of Gdańsk, Al. Gen. J. Hallera 107, PL-80-416 Gdańsk, Poland (e-mail: gorkos@gumed.edu.pl)

Generative reproduction traits of the perennial *Polygonatum multiflorum* (Asparagaceae) were studied in seven populations in 2000–2010. The frequencies of typical hermaphrodite flowers and functionally male flowers with short or atrophied carpels were 76%, 17% and 7%, respectively. Most hermaphrodite flowers occurred in the lower and middle positions on the ramets. The final fruit/flower ratio per ramet amounted to 25% and its variation was higher among populations than among years. The initial fruit set was on an average twice as high as the final fruit set. The mean number of seeds per fruit was 3.2, and the distribution was skewed to the right. At the inter-population level, the seed number and mass of fruits were negatively correlated with each other. Seed size/number trade off was significant for shoots and for fruits. The seedling emergence was 65%–82% and it was positively correlated with the seed size as well as the survival of seedlings. In the garden conditions, a few individuals flowered in the seventh year of development.

Introduction

The study of individual life histories in local plant populations provides a basis for predicting the future of these populations (Fröborg & Eriksson 1997, Silvertown & Charlesworth 2001, Verheyen *et al*. 2003). Important life history traits include the number and size of seeds, their germination and the development rate of juvenile individuals (Harper 1977). The fructification of allogamous clonal plants is often sparse. The hypotheses concerning the causes behind this phenomenon assume that parent plants make a limited investment in reproduction (Lloyd 1980). Most flowering plants are hermaphroditic but the investment in the male and female reproductive functions may vary considerably within and between individuals (Lloyd & Bawa 1984). The overproduction of flowers is explained by the adaptive character of this trait, which enables the pollen amount to increase, pollinators to be attracted and the number and quality of ovules to be optimised (Lloyd 1980).

Seed size is one of the most significant features connected with the adaptive ability of plants (Stearns 1992). It is often positively correlated with the emergence, size and persistence of seedlings (Turnbull *et al*. 2000, Moles & Westoby 2004), which may affect their recruitment (Eriksson 1995, Fröborg & Eriksson 1997). There are scarce experimental study data on the relationship between seedling emergence and

survival and seed size (Eriksson 1999, Lehtilä & Ehrlén 2005).

The population abundance of many clonal species is characterised by low dynamics, which results from their iterative growth and longevity (Eriksson & Ehrlén 2006). Seed production, germination and establishment are at least frequent enough to replace those individuals that die (Bierzychudek 1982). Even incidental recruitment of seedlings may balance the long-term downward trend and maintain genetic diversity (Eriksson 1997).

A number of forest species which occur only in ancient forests are characterised by a limited capability for colonising younger forests, on local and regional scales (Honnay *et al.* 2002). This might be due to the fact that in perennial forest species, such as *Polygonatum multiflorum*, the availability of seeds and ability to disperse them are limited (Ehrlén *et al.* 2006). As a result of forestry activities, ancient forests are fragmented, which leads to the formation of small, isolated plant populations. It is often observed that the plants' reproductive success and colonisation ability are reduced there (Jacquemyn *et al.* 2002, Kolb 2005). The continuity of the environment may allow a population to survive in hedgerows (Wehling & Diekmann 2009), but only under conditions similar to their natural habitats. The fact that local populations are endangered results from the insufficient number of seeds, low seed quality and dispersal, low recruitment of seedlings and poor persistence of juvenile individuals (Fröborg & Eriksson 1997, Jacquemyn *et al.* 2003).

Seed abundance and environmental factors have a greater effect on the seedling emergence than on the growth and survival to adult stages. The results of short-term studies are usually insufficient to predict these processes (Ehrlén *et al.* 2006, Kolb & Barsch 2010).

The present results were obtained during long-term studies of several *Polygonatum multiflorum* populations. It is a clonal perennial, fairly widespread and usually occurring in large numbers in the European forests. It was hypothesized that: (a) there is an annual inflow of seeds in the populations under study, (b) the survival of seedlings depends on the seed size, (c) juvenile individuals grow up until flowering. The objective of this work was to estimate the size and effectiveness of generative reproduction in *Polygonatum multiflorum*, especially: (1) sex allocation in flowers, (2) dynamics of fruit setting in populations, (3) seed number and size, and (4) seedling emergence and survival in field experiments.

Material and methods

Plant species

Polygonatum multiflorum (Asparagaceae) has a broad ecological scale (Ellenberg *et al.* 1992) and is widespread in the deciduous ancient forests (Hermy *et al.* 1999) of almost the entire Europe. It is a perennial herb which spreads vegetatively through a branching rhizome. Its aboveground shoots (ramets) consist of a simple stem, 30–80 cm tall, and alternating leaves. Axillary peduncles with 2–6 flowers grow in the leaf axils of the generative shoot. Flowering occurs sequentially from the bottom to the top of the shoot. Its greenish-white, tubular perianth is 9–20 mm long. The flowers are usually hermaphrodite, self-incompatible and insect-pollinated. There are six stamens in each flower and a simple pistil with a three-locular ovary and a linear style. The fruit is a bluish-black berry with endochorous seeds (DeFilipps 1980). The seeds do not form a persistent soil seed bank (Cerabolini *et al.* 2003), and the seedling development takes two years (*see* Ehrlén *et al.* 2006).

The nomenclature of the species follows Tutin *et al.* (1964–1980).

Study sites

The studies were conducted in seven populations located within a radius of 70 km from Gdańsk (N Poland) in 2000–2010. The populations were located in two regions: the Baltic shoreland, populations: Piaśnickie Łąki (PLA), Dębki (DEB), Sobieszewo (SOB), Junoszyno (JUN) and Stegna (STE), and the Pomeranian Lakeland, populations: Kloc (KL) and Ryjewo (RY). They were situated in forest areas measuring from 2 to 16 ha. The characteristics and abbreviations of the particular study sites are in Table 1. Informa-

tion on the soil conditions was based on chemical analyses performed at the National Chemical Agriculture Station in Warsaw.

Fruit setting and seed output

The studies on the generative reproduction of *P.* *multiflorum* began in 2000 in the PLA population, and in the other populations in the years that followed: DEB in 2002, KL and RY in 2005, JUN and SOB in 2006 and STE in 2007. Depending on the size of the population area, one to four patches were selected in each.

Every year in May/June, all the generative shoots were counted and the stem height, number

Table 1. The seven study sites with information on the areas of populations (m²), average density of *P. multiflorum* patches (generative shoots m⁻²), soil conditions and the dominant plant species. Information on the soil conditions was based on chemical analyses performed at the National Chemical Agriculture Station, Warsaw.

Site	Area (m²)	Density (shoots m⁻²)	Soil conditions	Dominant species	
				Forest stand	Ground cover
Piaśnickie Łąki (PLA)	25	5	damp, organic in soil, acidic, rich potassium and magnesium	*Quercus robur, Pinus silvestris, Betula pendula, Populus tremula, Sorbus aucuparia, Frangula alnus*	*Convallaria majalis, Maianthemum bifolium, Calamagrostis* sp., *Trientalis europaea, Molinia caerulea, Rubus* sp.
Dębki (DEB)	13	5	fresh, mineral-organic soil, acidic, moderately nutrient rich	*Quercus robur, Alnus glutinosa, Corylus avellana, Sorbus aucuparia*	*Anemone nemorosa, Oxalis acetosella, Ranunculus ficaria, Maianthemum bifolium, Paris quadrifolia, Rubus* sp.
Sobieszewo (SOB)	15	6	fresh, mineral, sandy soil, acidic, poor in magnesium and nitrogen	*Quercus robur, Tilia cordata, Betula pendula, Acer pseudoplatanus, Fraxinus excelsior, Sorbus aucuparia*	*Deschampsia flexuosa, Melampyrum pratense, Veronica* sp., *Lysimachia nemorum*
Junoszyno (JUN)	2	10	fresh, mineral, sandy soil, acidic, rich in potassium but poor in magnesium and nitrogen	*Pinus silvestris, Quercus robur, Acer platanoides*	*Vaccinium myrtillus, Oxalis acetosella, Maianthemum bifolium, Rubus saxatilis, Deschampsia flexuosa*
Stegna (STE)	12	18	fresh, mineral, sandy soil, less acidic, the most rich in nitrogen and calcium	*Quercus robur, Betula pendula, Acer pseudoplatanus, Sorbus aucuparia*	*Poa* sp., *Geranium robertianum, Impatiens parviflora, Maianthemum bifolium*
Kloc (KL)	2	25	fresh, mineral, sandy and clay soil, acidic, rich in potassium but poor in magnesium and nitrogen	*Quercus petraea, Alnus glutinosa, Frangula alnus, Sorbus aucuparia*	*Rubus* sp., *Pteridium aquilinum, Maianthemum bifolium, Mycelis muralis*
Ryjewo (RY)	20	10	fresh, mineral, sandy and clay soil, the least acidic, poor in magnesium and nitrogen	*Tilia cordata, Carpinus betulus, Quercus robur*	*Anemone nemorosa, Lamiastrum galeobdolon, Galium odoratum, Oxalis acetosella, Stellaria holostea, Hepatica nobilis, Maianthemum bifolium*

of leaves, flowers, and flower positions on the shoot were recorded in each patch. In addition, over the next two years, the number and location of young fruits on the marked ramets in all the populations were noted in July.

In September, ripe fruits were picked and the height of fruit position on the shoots was recorded. The measurements were performed on 986 shoots with ripe fruits (3661 fruits in total) where the seeds were counted. Air-dried seeds were weighed individually (5857 seeds) or as a total from each fruit (4799 seeds) to the nearest 0.1 mg.

The fruit-setting rate per shoot was calculated as the ratio of the number of young (initial fruit set) or ripe (final fruit set) fruits to the number of flowers. The position of flower/fruit on a shoot was classified in relative terms as lower, middle or upper (*see* Kudo *et al*. 2001).

Sex allocation in flowers

In 2008, in order to estimate the fraction of flowers potentially able to set fruit, 30 shoots were selected from each of the five populations, and 2817 flowers were collected from them. The material was kept in 70% ethanol in test tubes labelled with the shoot number and the height of flower position on the shoot until the morphological studies began.

The number of stamens and size of style were noted for each flower. The style of the carpel in typical (hermaphrodite) flowers has a stigma located at the level of the anthers. Some flowers had a carpel with a short style and a stigma below the anthers. There were some flowers with a hardly observable, atrophied carpel, and no ovules were developed in the ovary (male flowers). The number of ovules was determined in 260 flowers under a stereomicroscope (three shoots from each of the five populations).

Seedling establishment and plant development

The seed sowing experiments were performed to study the ontological development of the plants formed from seeds and the survival rate

of the seedlings and juvenile individuals. Seeds from the PLA population from 2002 were sown under natural conditions and in an experimental garden.

Seeds from 31 shoots were sown in the garden, 1–15 seeds into each separate 5-cm diameter peat container with sieved soil from the natural population sites (170 seeds in total). The containers were placed in soil and covered with natural leaf litter.

The seeds were sown under natural conditions with a view to studying the relationship between the seedling performance and seed size. They were sorted into four seed size categories: ≤ 18.0 mg, 18.1–22.0 mg, 22.1–30.0 mg, and ≥ 30.1 mg. Four to thirteen seeds ($n = 40$ groups) belonging to each of those categories (350 in total) were placed in sieved soil inside a wooden frame.

In the second year, the number of seedlings (leafy stage) was determined after each sowing. In the following years, the presence of new seedlings (timing of seeds) as well as the number and size of the juvenile ones were recorded.

In the autumn of 2007 and 2008, observations of the size and architecture of the individuals were conducted after the rhizomes of juvenile plants from the seeds sown in 2002 had been partially exhumed.

In case of post-dispersal seed predation (*see* Eriksson 1999), which may affect the assessment of seedling emergence, and in order to check whether buried seeds would germinate, the seeds were sown into metal-net containers (10 cm high and 5 cm in diameter) in 2004. Fifteen to sixteen seeds were placed in each of the two containers with sieved soil from the natural habitat of the PLA population at a depth of 5 cm and 10 cm, 63 seeds in total. The containers were buried vertically, their top edge at the soil surface, and covered with leaf litter. After two years they were exhumed, and the seedlings were counted and removed. The remaining ungerminated seeds were reburied and controlled in the next year.

Statistical analyses

The normality of the frequency distributions of the values was estimated with Shapiro-Wilk's test.

For the traits whose distribution was significantly different from normal (number of ovules in ovaries, seeds in fruits, seed mass) the differences between samples were tested with the Mann-Whitney (M-W) *U*-test. Yearly dynamics and variation in fruit production, number of seeds, seed mass among populations, seedling emergence and survival were analyzed with the Kruskal-Wallis (K-W) test. The dependencies between shoot height, number of ovules, seeds, seed mass, seedling emergence and survival were determined with Spearman's rank correlation (r_S). The seed size/number trade-off was also determined with a partial correlation coefficient (Sokal & Rohlf 1995). All statistical analyses were conducted with Statistica ver. 8.0 (StatSoft Inc. 2008).

Results

Sex allocation among and within plants

In each of the studied flowers from the five *P. multiflorum* populations, there were six stamens with anthers that were morphologically typical of this species. Typical carpels contained one to four ovules in each locule. Atrophied carpels did not have ovules in the ovaries at all, and carpels with short styles (86%) mostly had no ovules.

In the populations, the frequency of flowers with typical, short and atrophied styles was 76%, 17% and 7% on the average, respectively. Only in one population (KL) the percentage of hermaphrodite flowers with typical carpels was significantly higher than in the other four populations: SOB, DEB, RY and PLA (K-W: $H_{4,145} = 27.7$, $p < 0.001$; Table 2).

In total, 23% of the shoots from the five populations had flowers only with typical carpels. A

small percentage of the shoots (10%), with small numbers of leaves (8–14) and flowers (1–12), had flowers exclusively with short and atrophied styles. On most of the shoots (67%), flowers with both typical shortened and atrophied styles were found. The size of the flower fraction with typical carpels correlated weakly with the features determining ramet size, i.e. with leaf and flower numbers ($r_S = 0.24$ and 0.26, respectively, $p < 0.01$).

Most of the hermaphrodite flowers, with typical carpels, occurred in the lower and middle positions (41% and 46%, respectively), while only 13% grew in the upper one. The majority of the male flowers with atrophied carpels were found in the upper position (83%), and the rest in the middle (14%) and rarely lower positions (3%). Similarly, most of the flowers with short carpels were in the upper shoot positions (54%), whereas the remaining flowers in the middle and lower positions (39% and 7%; Fig. 1).

The number of ovules in a typical carpel was 9.5 ± 2.3 (mean ± SD), and the values of this trait were significantly different between the populations under study (K-W: $H_{4,257} = 35$, $p < 0.001$). Moreover, the number of ovules in the flowers located in the lower, middle and upper shoot positions differed considerably (10.6 ± 1.6, 9.5 ± 2.0, and 6.4 ± 3.0 respectively; K-W: $H_{2,257} = 77.7$, $p < 0.001$; Fig. 2).

Fruit setting and seed output

Every year, the number of fructifying shoots in each of the studied populations was smaller than the number of flowering ones. Two populations had the highest dynamics of the fructifying shoot fraction: PLA (4%–69%) and RY (3%–60%).

Table 2. Number of flowers per shoot and number of ovules per flower (mean ± SD) of *Polygonatum multiflorum* from five populations.

Population	Number of shoots	Flowers per shoot	Hermaphrodite flowers (%)	Number of ovaries	Number of ovules
SOB	33	22.6 ± 16.0	59.6	40	10.4 ± 2.2
DEB	31	16.9 ± 14.0	56.7	27	9.2 ± 2.1
RY	37	15.0 ± 12.1	66.7	38	10.6 ± 1.7
KL	25	26.1 ± 18.8	89.7	84	8.7 ± 3.6
PLA	17	18.5 ± 15.3	74.0	78	8.4 ± 2.7
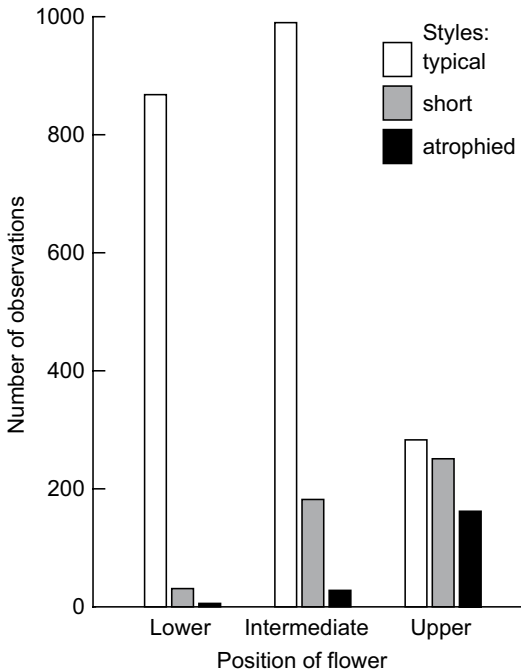
Fig. 1. Number of flowers of *Polygonatum multiflorum* with typical, short and atrophied styles in different flower positions on the shoot.
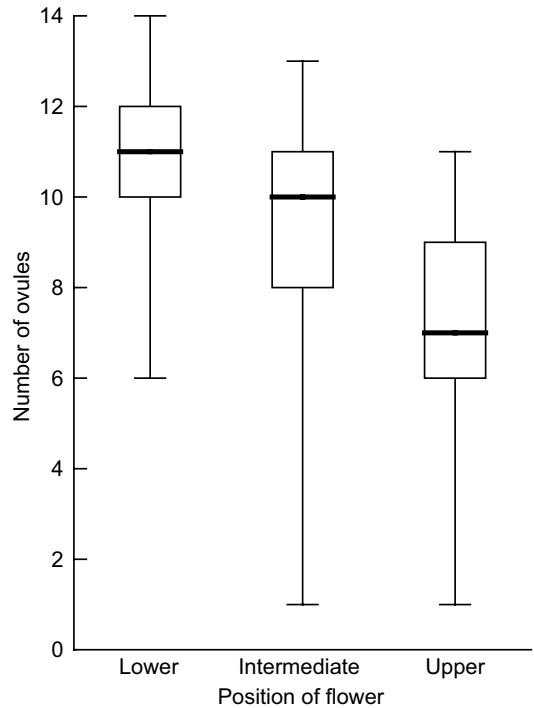

Fig. 2. Number of ovules per ovary of *Polygonatum multiflorum* for different flower positions (median, 25% and 75% quartiles, and range). Values differ significantly (M-W: $U = 338$, df = 1, $p < 0.05$).

In the others, the differences were also considerable: KL (9%–50%), SOB (8%–46%), JUN (23%–83%), DEB (35%–80%) and STE (40%–67%). The substantial dynamics of the fructifying shoots usually resulted from damage caused by grazers (primarily roe deer) and *Phymatocera aterrima* (Hymenoptera) larvae, the eggs of which, laid under the epidermis of *P. multiflorum* stems, were observed in the flowering period. In addition, in each of the populations the lack of fruits on shoots was due to the fact that flowers, mainly from the short shoots, were shed after anthesis.

The number of flowers on a shoot was 20.5 ± 12 (mean ± SD, range = 1–69), and it differed significantly among the populations (K-W: $H_{6,1066} = 303$, $p < 0.001$), while the variability among years was smaller (K-W: $H_{10,1066} = 62$, $p < 0.001$). Floral display (number of flowers which bloomed on a ramet at the same time) was on average (median) three, and its only slightly differed among the populations (K-W: $H_{4,144} = 16.4$, $p < 0.01$). The flowers were predominantly visited by bumble-bees (*Bombus* sp.).

The fruit/flower ratio (fruit set) per ramet amounted to 24.5% and was very variable (coefficient of variation, CV = 84.6%). The long-term dynamics of the fruit set played an important role in this variability (Fig. 3). However, the fruit-set variation was higher among populations than among years (K-W: $H_{6,962} = 236$, and $H_{10,962} = 32$, $p < 0.001$, respectively). The percentage of fruit set was weakly negatively correlated with ramet size, expressed as the plant height and number of leaves and flowers ($r_S = –0.27$, $–0.3$, and $–0.37$, respectively). The proportion of flowers that set fruit was higher on the lower and middle positions than on the upper ones, being 42%, 47% and 11%, respectively. The initial fruit/flower ratio (for young fruits) was on the average twice as high as the final fruit set (for ripe fruits) and was calculated at 45.7% as compared to 20.8%. This difference was the largest in the PLA population (80% as compared with 10% in 2008), and the proportions of young and ripe fruits in RY were similar in 2007. The initial fruit set was
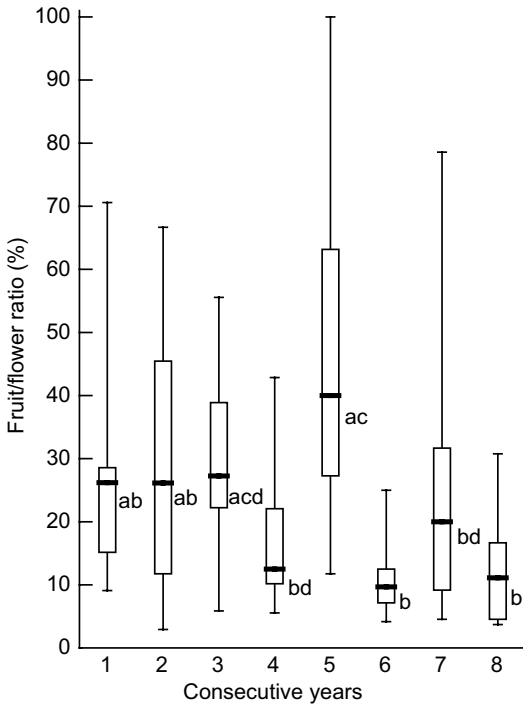
Fig. 3. Long-term variation of fruit to flower number ratio of *Polygonatum multiflorum* in the Dębki population (median, 25% and 75% quartiles, and range). Boxes with the same letter do not differ (K-W: $p > 0.05$).
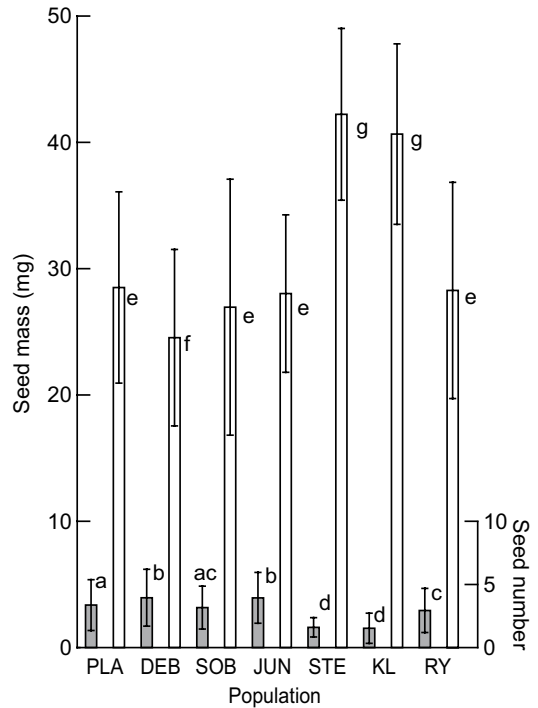
Fig. 4. Seed mass (open bars) per fruit and seed number (shaded bars) per fruit in seven populations of *Polygonatum multiflorum* (mean ± SD, $n = 3384$). Bars with the same letter do not differ (K-W: $p > 0.05$).

significantly positively correlated with the soil richness in nitrogen and magnesium (r_S was 0.81 and 0.87, respectively, $p < 0.05$; *see* Table 1).

Fruits with aborted seeds were found in all the populations, but their proportion was small, e.g. 1%–5% in the STE and 6%–14% in the JUN population. Fruits with seeds damaged by *Contarinia polygonati* (Diptera) larvae which fed on them were observed in each population. In some of the populations and years the percentage of fruits with damaged seeds was high, e.g. in PLA (54%), SOB (30%) and JUN (38%).

The mean number of seeds in a fruit was 3.2 ± 2.0 (range = 1–12), and the distribution of this trait was skewed to the right in all the populations (skewness from 0.5 to 3.7). The smallest number (mean ± SD) was recorded in the fruits of the populations KL (1.5 ± 1.2) and STE (1.6 ± 0.8), whereas the largest were in JUN (3.9 ± 2) and DEB (4 ± 2.3; Fig. 4). The variability of the seed number in a fruit was higher among populations than among years (K-W: $H_{6,3384} = 635$, and

$H_{10,3384} = 147$, $p < 0.001$). The mean seed number in a fruit was weakly negatively correlated with shoot size expressed as the number of flowers ($r_S = -0.12$, $p < 0.001$), and positively with the fruit set ($r_S = 0.33$, $p < 0.001$). The fruits located on the upper shoot positions had fewer seeds (K-W: $H_{2,2347} = 89.9$, $p < 0.001$).

Seed mass was 28.7 ± 8.6 mg (mean ± SD, range = 5–61 mg), and the distribution of this trait was slightly asymmetrical (Fig. 5). The heaviest seeds (mean ± SD) were in populations STE (41.7 ± 7.5 mg) and KL (40.9 ± 7.3 mg), while the lightest were in DEB (24.1 ± 6.5 mg; Fig. 4). Even though the mean mass of the seeds changed significantly over the years in each population, its variability was higher among the populations than among the years (K-W: $H_{6,5857} = 1950$, $p < 0.001$, and $H_{10,5857} = 387$, $p < 0.001$). The mean seed mass on a shoot was weakly positively correlated with shoot height and number of leaves and flowers ($r_S = 0.18$, 0.14 and 0.25, respectively, $p < 0.001$), and negatively with the
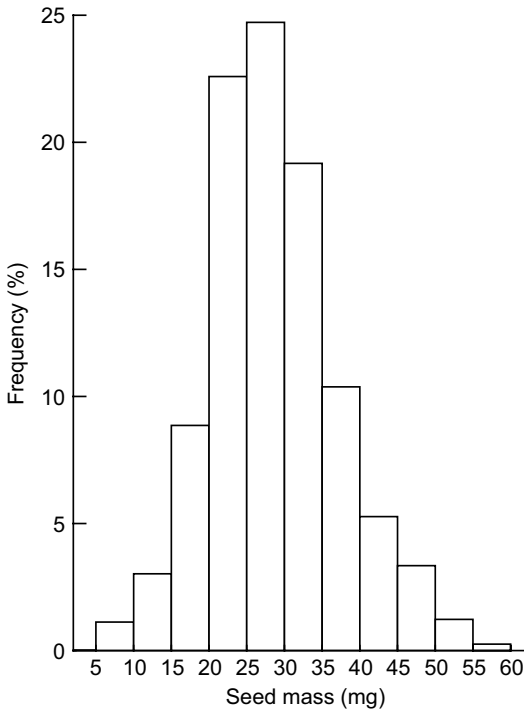
Fig. 5. Frequency distribution of *Polygonatum multiflorum* seed mass. The distribution was based on 5857 individually weighed seeds from seven populations. The seed mass was 28.7 ± 8.6 mg (mean ± SD) and the skewness was 0.41.
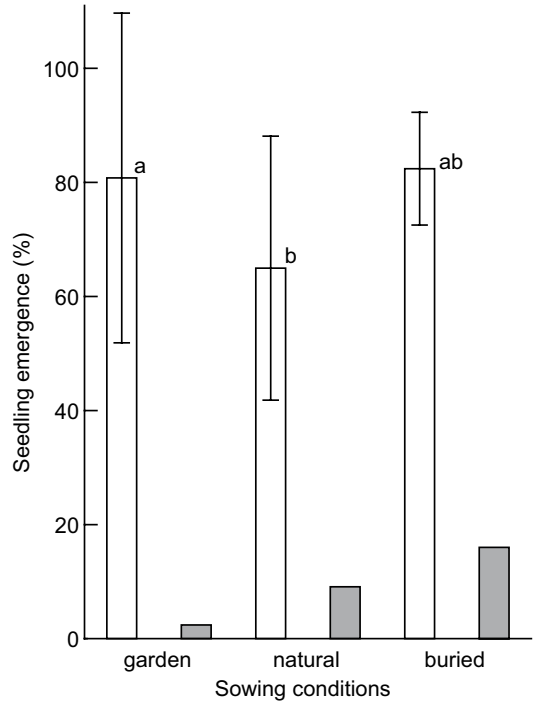
Fig. 6. Seedling emergence (mean ± SD) in the second (white bars) and third years (grey bars) after sowing *Polygonatum multiflorum* seeds in the garden, natural conditions and deep sowing. Bars with the same letter do not differ (K-W: $p > 0.05$).

fruit set on a shoot ($r_S = -0.31$). The mean seed mass in a fruit did not vary significantly with the fruit position on a shoot (K-W: $p = 0.13$).

On an inter-population level, the seed number and mass in the fruits were negatively correlated with each other ($r_S = -0.86$; Fig. 4). Moreover, the seed size/number trade-off was significant for the shoots and for the fruits ($r_S = -0.20$ and -0.32, respectively, $p < 0.001$). Partial correlations, when the total seed mass on a shoot and in a fruit was controlled, were stronger and amounted to $r = -0.56$ and $r = -0.82$, respectively.

Seedling establishment and plant development

Seedling emergence was 65%–82% in the second year after the sowing of seeds from the PLA population. Moreover, some seeds (2%–16%) gave rise to seedlings in the third year, after timing (Fig. 6).

In the garden, the seedling emergence was high (arithmetic mean 81%, median = 100%) and varied for the seeds from different ramets (CV = 0.36). The size of the seed fraction which formed seedlings was significantly correlated with the mean seed mass ($n = 31$, $r_S = 0.49$, $p < 0.01$). The seedling survival until the next year (juvenile stage) was very high (99%), but in the following years it decreased down to 42% in the seventh year (Fig. 7). Two-year-old individuals had one leaf each, three-year-old ones had 2–3 leaves, and five-year-old ones had 4–6 leaves. In the seventh year of development the individuals had 1–12 leaves, but in the largest fraction (28%) three leaves were found. The highest observed number of leaves (10 and 12) was noted in two 7-year-old individuals, which had 2 and 4 flowers for the first time, but did not set fruit.

In the natural environment, the mean seedling emergence was 65%, (CV = 0.36). The size of the seed fraction where seedlings were formed

was positively correlated with the mass of seeds sorted into four groups ($n = 40$, $r_S = 0.43$, $p < 0.01$). The survival of juvenile individuals was about 80% up to the fifth year. A significant decrease of the survival rate to 56% took place in the seventh year (Fig. 7). In each year, the survival of individuals was positively correlated ($r_S = 0.3$, $p < 0.05$) with the mass of seeds which they grew from. Two-year-old individuals had 1–3 leaves, three-year-old ones had 4, and five-year-old ones had 5–7 leaves. In the seventh year, the individuals had 1–8 leaves. The largest fraction (35%), however, had only one leaf. After four vegetation seasons, in autumn, the majority (76%) of juvenile individuals had rhizomes with primary roots. The others had lost 1–2 oldest annual increments, and just few (4%) had rhizome lateral buds. After five years of growth two-shoot individuals were found (previous-year lateral buds had formed branched rhizomes). Only 25% of the individuals had an entire rhizome, while the others had disintegrated rhizomes.

Seedling emergence from the seeds sown into containers buried in soil was 82%. In the following year 91% of the remaining seeds (16% timing) produced seedlings. It follows that in the third year after sowing 98% of the seeds in total gave rise to seedlings.

Discussion

Sex allocation

Above-ground *P. multiflorum* shoots are characterised by a large number of sequentially and acropetally developing flowers. A morphological and functional variation of flowers is often observed in such plants (Brunet & Charlesworth 1995, Kudo *et al.* 2001, Ishii & Sakai 2002, Guitian *et al.* 2004). On the *P. multiflorum* shoots, beside the hermaphrodite flowers and functionally male flowers, there were flowers with short styles. Such a form of andromonoecy may be an evolutionary tendency to reduce the female function allocation and preserve the male function (Spalik 1991, Brunet & Charlesworth 1995).

The studied *P. multiflorum* populations differed in the hermaphrodite flower fraction, the same as in *P. odoratum* (Guitian *et al.* 2004). The
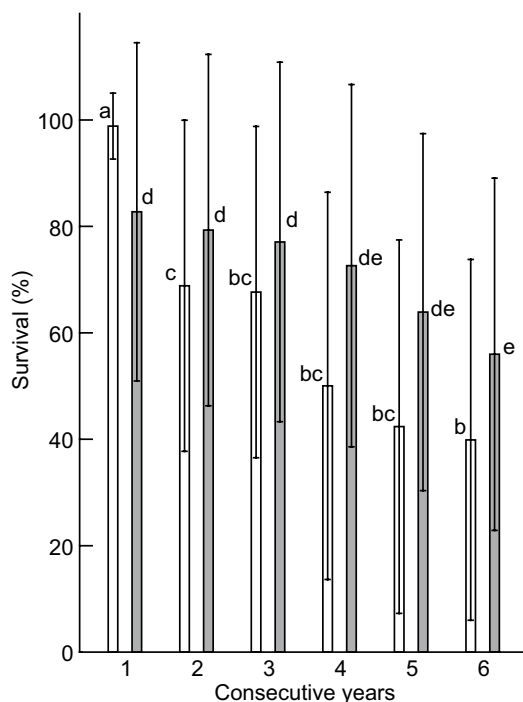
Fig. 7. Survival (mean ± SD) of *Polygonatum multiflorum* individuals emerged from seeds sown in the garden (white bars) and natural conditions (grey bars). Bars with the same letter do not differ (K-W: $p > 0.05$).

proportion of hermaphrodite flowers was smaller in the upper shoot positions, which has also been observed in *P. odoratum*, *Corydalis ambigua* and *Narthecium asiaticum* (Kudo *et al.* 2001, Ishii & Sakai 2002, Guitian *et al.* 2004).

Among the populations, the variation in the number of ovules in *P. multiflorum* flowers was significant contrary to the one observed in *P. odoratum* (Guitian *et al.* 2004). The number of ovules, however, decreased from the proximal to the distal position of *P. multiflorum* flowers, which has also been recorded for *P. odoratum* (Guitian *et al.* 2004), *Narthecium asiaticum* (Ishii & Sakai 2002) and *Lobelia sessiliflora* (Hiraga & Sakai 2007). Sex allocation theories assume that a change in sex allocation leads to a change in reproductive success (Lloyd & Bawa 1984, Brunet & Charlesworth 1995).

Fruit setting and seed output

Fruit production is limited by a number of not

only environmental factors but also those resulting from the competition for resources within a plant during its consecutive development stages (Lloyd 1980). In *P. multiflorum*, fruits were set from a much smaller flower fraction than the proportion of hermaphrodite flowers would suggest. Furthermore, the distribution of resources to ovaries that matured into fruits on *P. multiflorum* shoots may not be uniform. The negative correlation between the fruit set and shoot size might be the outcome of this phenomenon.

The variation of fruit set among populations of *Primula elatior* for example was interpreted as a result of varied pollen limitation among the populations that differed in size (Jacquemyn *et al.* 2001). The lack of correlation between the fruit set and population abundance of *P. multiflorum* may stem from temporal variation of herbivore pressure (insects, molluscs, mammals). Higher variability of the fruit set among populations than among years probably resulted from the effect of local environmental (amount of soil N and Mg) and intra-population factors (density).

The mean fruit set was three times lower than that observed in *P. odoratum* (Guitian *et al.* 2001), similar to that recorded for *Convallaria majalis* (Eriksson 1999) and twice as high as in *Maianthemum bifolium* (Kosiński 2008), which might be due to the longer fruit exposure in *M. bifolium* (Eriksson & Ehrlén 1991) and consequently the greater risk of losing fruits through predation.

The results of the experimental studies by Guitian *et al.* (2001) on *Polygonatum odoratum* showed that fruits are more often set at proximal than at distal flower positions because of competition for resources rather than due to pollen limitations or architectural effects. *Polygonatum multiflorum* shoots also had a higher fruit set in the proximal than in the distal flowers. The decreasing number of seeds (and their total mass) from the bottom to the top of a shoot may result from resource limitation during acropetal flower development, just as in *P. odoratum* (Guitian *et al.* 2001).

The seed size/number trade-off is a result of allocation of a relatively fixed proportion of plant resources for seed development (Shipley & Dion 1992). The mean seed mass was negatively related to the seed number per plant, as in *Banksia marginata* (Vaughton & Ramsey 1998) and *Lupinus polyphyllus* (Aniszewski *et al.* 2001). Seed mass and number relation per *P. multiflorum* fruit was lower than that reported by Eriksson and Ehrlén (1991) for 34 fleshy-fruit and vertebrate-dispersed species ($r = -0.74$), but higher than in *M. bifolium* ($r = -0.16$; Kosiński 2008) and *P. quadrifolia* ($r = -0.25$; Kosiński 2010). Serious herbivore-inflicted damage to the fruits undoubtedly weakened the relationships between features of the shoot, fruit and seed size.

Seedling establishment and plant development

Long-term observations of the results of seed sowing experiments are of especial value because in many species the demands for establishment are more stringent than for germination (Turnbull *et al.* 2000). The results of such studies may be highly year- and site-dependent, and hence ought to be repeated in different years and sites (Eriksson 1997, Turnbull *et al.* 2000). The results of the studies conducted by Ehrlén *et al.* (2006) show that a bottleneck at post-seedling stages makes short-term assessments poor predictors of recruitment success over long time.

My results of several-year seed-sowing experiments indicate a high *P. multiflorum* seedling emergence both under natural conditions and in the garden. In addition, the sowing shows timing in seed germination, which was also observed in *Convallaria majalis* (Eriksson 1999). High seedling emergence (98%), recorded after deep sowing, may suggest that values of this trait were underestimated after sowing onto an open soil surface, when the seeds are exposed to post-dispersal predation (Eriksson & Ehrlén 1991). Furthermore, the results of my experiment indicate high seedling emergence of the buried seeds, which is typical of large seeds (Jankowska-Błaszczuk & Daws 2007).

The survival rate of juvenile *P. multiflorum* individuals decreased year by year. Nevertheless, the observed per seed response of juvenile individuals was much stronger under natural conditions than Baeten *et al.* (2009) found in their studies of recent forests.

Only a small fraction of *P. multiflorum* individuals reached the generative stage. Under natural conditions the same-aged plants were smaller and vegetative. Ehrlén *et al*. (2006) found that the plants flowered after 11 years of observations in a single patch.

My own results of seed-sowing experiments show a positive relationship between seed size and both seedling emergence and survival under natural conditions and in the garden. The same relationship was observed for example in *Arum italicum* (Mendez 1997), *Primula elatior* (Jacquemyn *et al*. 2001), *Primula veris* (Lehtilä & Ehrlén 2005) and *Maianthemum bifolium* (Kosiński 2008). In the studies on a group of forest species (among others *P. multiflorum*) carried out by Ehrlén and Eriksson (2000), it was found that both emergence rate and survival until the third year were positively correlated with seed size. As for my observations of *P. multiflorum*, this relationship persisted for seven years.

Seedlings were present in the areas of the *P. multiflorum* populations under study (unpublished data). However, the estimation of the natural seedling emergence requires exhuming the plants and causing damage to them. Single-leaf juvenile individuals that are several years old are also plentiful in the populations. Their proportion increases as a result of damage to young plants (done mainly by molluscs), which results in the regression of the number of leaves in the following year. This phenomenon was also observed in experimental sowing, where some of the single-leaf shoots were branches of rhizomes belonging to five-year-old individuals formed from seeds.

My results of studies on seven populations showed a high variability in seed production. Although the level of seed production per ramet was low, the seed production in populations was considerable, which results predominantly from the abundance of fructifying ramets. Large seed output combined with high germination ability and substantial survival of the seedlings can provide a yearly inflow of genets to the populations as long as the suitable microsites are available within the population area (Eriksson 1995). The long-lasting process of reaching the generative stage is estimated at over ten years (Ehrlén *et al*. 2006).

As a result of experimental sowing of seeds produced in one year (on 31 shoots), a population of 65 individuals, two of which grew to generative maturity, was formed after nine years. In the years to come, the juvenile individuals have a chance of approaching maturity after crossing the body-size threshold, since mortality decreases with body size (Eriksson 1995). In future research, I intend to compare the genetic diversity in natural populations and in populations formed by experimental sowing.

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References

Aniszewski, T., Kupami, M. H. & Leinonen, J. 2001: Seed number, seed size and seed diversity in Washington lupin (*Lupinus polyphyllus* Lindl.). — *Annals of Botany* 87: 77–82.

Baeten, L., Jacquemyn, H., Van Calster, H., Van Beek, E., Devlaeminck, R., Verheyen, K. & Hermy, M. 2009: Low recruitment across life stages partly accounts for the slow colonization of forest herbs. — *Journal of Ecology* 97: 109–117.

Bierzychudek, P. 1982: Life histories and demography of shade-tolerant temperate forest herbs: a review. — *New Phytologist* 90: 757–776.

Brunet, J. & Charlesworth, D. 1995: Floral sex allocation in sequentially blooming plants. — *Evolution* 49: 70–79.

Cerabolini, B., Ceriani, R. M., Caccianiga, M., De Andreis, R. & Raimondi, B. 2003: Seed size, shape and persistance in soil: a test on Italian flora from Alps to Mediterranean coasts. — *Seed Science Research* 13: 75–85.

DeFilipps, R. A. 1980: *Polygonatum* Miller. — In: Tutin, T. G. (ed.), *Flora Europaea* 5: 70–71. Cambridge University Press, Cambridge.

Ehrlén, J. & Eriksson, O. 2000: Dispersal limitation and patch occupancy in forest herbs. — *Ecology* 81: 1667–1674.

Ehrlén, J., Munzbergova, Z., Diekmann, M. & Eriksson, O. 2006: Long-term assessment of seed limitation in plants: results from an 11-year experiment. — *Journal of Ecology* 94: 1224–1232.

Ellenberg, H., Weber, H. E., Düll, R., Wirth, V., Werner, W. & Paulißen, D. 1992: Indicator values of plants in central Europe. — *Scripta Geobotanica* 18.

Eriksson, O. 1995: Seedling recruitment in deciduous forest herbs: the effect of litter, soil chemistry and seed bank.

— *Flora* 190: 65–70.

Eriksson, O. 1997: Clonal life histories and the evolution of seed recruitment. — In: de Kroon, H. & van Groenendael, J. (eds.), *The ecology and evolution of clonal plants*: 211–226. Backhuys, Leiden.

Eriksson, O. 1999: Seed size variation and its effect on germination and seedling performance in the clonal herb *Convallaria majalis*. — *Acta Oecologica* 20: 61–66.

Eriksson, O. & Ehrlén, J. 1991: Phenological variation in fruit characteristics in vertebrate-dispersed plants. — *Oecologia* 86: 463–470.

Eriksson, O. & Ehrlén, J. 2006: Long-term dynamics of herb populations in Swedish deciduous forests: some generalizations. — *Polish Botanical Studies* 22: 185–194.

Fröborg, H. & Eriksson, O. 1997: Local colonization and extinction of field layer plants in a deciduous forest and their dependence upon life history features. — *Journal of Vegetation Science* 8: 395–400.

Guitian, J., Guitian, P. & Medrano, M. 2001: Causes of fruit set variation in *Polygonatum odoratum* (Liliaceae). — *Plant Biology* 3: 637–641.

Guitian, J., Medrano, M. & Oti, J. E. 2004: Variation in floral sex allocation in *Polygonatum odoratum* (Liliaceae). — *Annals of Botany* 94: 433–440.

Harper, J. L. 1977: *Population biology of plants*. — Academic Press, London.

Hermy, M., Honnay, O., Firbank, L., Grashof-Bokdam, C. & Lawesson, J. E. 1999: An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. — *Biological Conservation* 91: 9–22.

Hiraga, T. & Sakai, S. 2007: The effects of inflorescence size and flower position on biomass and temporal sex allocation in *Lobelia sessiliflora*. — *Plant Ecology* 188: 205–214.

Honnay, O., Verheyen, K., Butaye, J., Jacquemyn, H., Bossuyt, B. & Hermy, M. 2002: Possible effects of habitat fragmentation and climate change on the range of forest plant species. — *Ecology Letters* 5: 525–530.

Ishii, H. S. & Sakai, S. 2002: Temporal variation in floral display size and individual floral sex allocation in racemes of *Narthecium asiaticum* (Liliaceae). — *American Journal of Botany* 89: 441–446.

Jacquemyn, H., Brys, R. & Hermy, M. 2001: Within and between plant variation in seed number, seed mass and germinability of *Primula elatior*: effect of population size. — *Plant Biology* 3: 561–568.

Jacquemyn, H., Brys, R. & Hermy, M. 2002: Patch occupancy, population size and reproductive success of a forest herb (*Primula elatior*) in a fragmented landscape. — *Oecologia* 130: 617–625.

Jacquemyn, H., Butaye, J. & Hermy, M. 2003: Influence of environmental and spatial variables on regional distribution of forest plant species in fragmented and changing landscape. — *Ecography* 26: 768–776.

Jankowska-Błaszczuk, M. & Daws, M. I. 2007: Impact of red: far red ratios on germination of temperate forest herbs in relation to shade tolerance, seed mass and persistence in the soil. — *Functional Ecology* 21: 1055–1062.

Kolb, A. 2005: Reduced reproductive success and offspring survival in fragmented populations of the forest herb *Phyteuma spicatum*. — *Journal of Ecology* 93: 1226–1237.

Kolb, A. & Barsch, K. 2010: Environmental factors and seed abundance influence seedling emergence of a perennial forest herb. — *Acta Oecologica* 36: 507–513.

Kosiński, I. 2008: Long-term variability in seed size and seedling establishment of *Maianthemum bifolium*. — *Plant Ecology* 194: 149–156.

Kosiński, I. 2010: Long-term variation in seed mass and seed production in populations of *Paris quadrifolia*. — *Plant Biology* 12: 206–214.

Kudo, G., Maeda, T. & Narita, K. 2001: Variation in floral sex allocation and reproductive success within inflorescences of *Corydalis ambigua* (Fumariaceae): pollination efficiency of resource limitation? — *Journal of Ecology* 89: 48–56.

Lehtilä, K. & Ehrlén, J. 2005: Seed size as an indicator of seed quality: a case study of *Primula veris*. — *Acta Oecologica* 28: 207–212.

Lloyd, D. G. 1980: Sexual strategies in plants. I. An hypothesis of serial adjustment of maternal investment during one reproductive session. — *New Phytologist* 86: 69–79.

Lloyd, D. G. & Bawa, K. S. 1984: Modification of the gender of seed plants in varying conditions. — *Evolutionary Biology* 17: 255–338.

Mendez, M. 1997: Sources of variation in seed mass in *Arum italicum*. — *International Journal of Plant Sciences* 158: 298–305.

Moles, A. T. & Westoby, M. 2004: Seedling survival and seed size: a synthesis of the literature. — *Journal of Ecology* 92: 372–383.

Shipley, B. & Dion, J. 1992: The allometry of seed production in herbaceous Angiosperms. — *American Naturalist* 139: 467–483.

Silvertown, J. W. & Charlesworth, D. 2001: *Introduction to plant population biology*. — Blackwell, Oxford.

Sokal, R. R. & Rohlf, F. J. 1995: *Biometry*. — Freeman, New York.

Spalik, K. 1991: On the evolution of andromonoecy and 'overproduction' of flowers: a resource allocation model. — *Biological Journal of Linnaean Society* 42: 325–336.

Stearns, S. C. 1992: *The evolution of life histories*. — Oxford University Press, New York.

Turnbull, L. A., Crawley, M. & Rees, M. 2000: Are plant populations seed-limited? A review of seed sowing experiments. — *Oikos* 88: 225–238.

Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M. & Webb, D. A. (eds.) 1964-1980: *Flora Europaea*. — Cambridge University Press, Cambridge.

Vaughton, G. & Ramsey, M. 1998: Sources and consequences of seed mass variation in *Banksia marginata* (Proteaceae). — *Journal of Ecology* 86: 563–573.

Verheyen, K., Honnay, O., Motzkin, G., Hermy, M. & Foster, D. R. 2003: Response of forest plant species to land-use change: a life-history trait-based approach. — *Journal of Ecology* 91: 563–577.

Wehling, S. & Diekmann, M. 2009: Hedgerows as an environment for forest plants: a comparative case study of five species. — *Plant Ecology* 204: 11–20.