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Original Article

**Spatiotemporal variation in the pollination systems of a supergeneralist plant: Is *Angelica sylvestris* (Apiaceae) locally adapted to its most effective pollinators?**

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Running title: Spatiotemporal variation in the pollination systems of a supergeneralist umellifer

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## Abstract

**Background and Aims:** In terms of pollination systems umbellifers (plants of the Carrot family, Apiaceae) are regarded generalists, since their (usually dichogamous) flowers are visited by a wide range of insects representing several taxonomic orders. However, recent analyses of insect effectiveness revealed that these plants may be pollinated effectively by a narrow assemblage of insect visitors. We were interested: (1) whether different populations of umbellifer species exhibited any variation in pollinator assemblages (also over the course of several years) that could lead to local specialization of the pollination system, (2) whether such variation (if present) coincided with phenotypic variation (including nectar and scent characters), indicating the occurrence of co-evolutionary processes, and finally (3) whether it is adaptive, i.e., it resulted in increased reproductive output by populations.

**Methods:** In our study, we focused on *Angelica sylvestris* L., a common European species visited by a taxonomically diverse insect assemblage. In three populations, located along an approx. 700 km transect, over three growth seasons, we identified insect visitors, assessed their effectiveness by surveying pollen loads present on the insect body and insect activity on umbels, studied nectar and scent composition, and performed transplantation experiments.

**Key Results:** We showed that the populations investigated in this study differ in their nectar and scent profiles and, despite the similar taxonomic composition of insect visitor assemblages, were effectively pollinated by disparate pollinator morphogroups, i.e. flies and beetles. Although this suggested local adaptations to the most effective pollinators, our analyses demonstrated functional equivalency of the visitor morphogroups, which is probably related to the fact that *A. sylvestris* bears few ovules per flower. Our transplantation experiments confirmed that reproductive success was not related to the source of experiment plants and that the insects do not exhibit preferences towards local genotypes.

1 **Conclusions:** *Angelica sylvestris* is morphologically well adapted to ecological  
2 generalization, and that the observed variation in floral characters can be interpreted as  
3 “adaptive wandering”. Specialization in this family seems only possible under very special  
4 circumstances, for example, when the pollinator community comprises insect visitor groups  
5 that clearly differ in their pollination capacity. However, barrier to evolve any morphological  
6 improvements resulting in the fine-tuning of the flower towards particular pollinator types  
7 may well be the same as those architectural constraints on the floral bauplan that make  
8 umbellifers so uniform in their floral displays, and so successful in attracting large numbers of  
9 pollinators.

10  
11 **Key words:** adaptive wandering, cantharophily, floral scent, generalization, myophily, nectar  
12 amino acids, phenotype, specialization, umbel

## INTRODUCTION

Pollination by animals (zoogamy) prevails amongst angiosperms (Willmer, 2011), and it has long been proposed that this process is an important driver of angiosperm evolution (Grant, 1949; van der Niet and Johnson, 2012; Van der Niet *et al.*, 2014a; Brosi, 2016).

Entomophilous plants vary from specialists having a single or a small number of pollinators, and derived from a very narrow taxonomic group, to supergeneralists pollinated by a large number of taxonomically disparate species (Ollerton, 1996; Proctor *et al.*, 1996; Johnson and Steiner, 2000; Willmer, 2011). In fact, some authors have suggest that generalist pollination predominates in nature (Jordano, 1987; Herrera, 1996; Waser *et al.*, 1996; Reverté *et al.*, 2016) (for a somehow different view, however, see e.g. Ambruster *et al.*, 2000; Willmer, 2011; Padyšáková *et al.*, 2013; Bartoš *et al.*, 2015). One of the important arguments against obligate specialization is based on the evidence that plant-pollinator interactions are highly variable across time and space, both qualitatively or quantitatively (Schemske and Horvitz, 1984; Herrera, 1988; Herrera, 1989; Ollerton, 1996; Waser *et al.*, 1996; Gómez and Zamora, 1999; Price *et al.*, 2005; Gómez and Zamora, 2006; Cosacov *et al.*, 2008; Artz *et al.*, 2010; Castro *et al.*, 2013). There are, however, caveats to this reasoning, since most data published on the evolution of pollination systems is based on studies of relatively specialized plants (at least phenotypically), whereas information concerning phenotypical generalists, i.e. plants with flowers easily accessible for a wide taxonomic array of visitors, is relatively scarce (Ollerton *et al.*, 2007). Furthermore, co-evolutionary processes among plants and pollinators resulting in the macro-evolutionary diversity of angiosperms act at the population level (Armbruster, 1985; Thompson, 2005; Herrera *et al.*, 2006; Johnson, 2006; Johnson, 2010) with increasing evidence on the evolution of pollination ecotypes adapted to local pollinator assemblages (Johnson, 2006; Perez-Barrales *et al.*, 2007; Anderson *et al.*, 2009;

1 Armbruster and Muchhala, 2009; Gómez *et al.*, 2009a; Johnson, 2010; Cosacov *et al.*, 2014;  
2 Gomez *et al.*, 2014; Van der Niet *et al.*, 2014b; Yamada *et al.*, 2014). Therefore, as postulated  
3 by Thompson (2005), many plants that appear to be specialists at the population level, may in  
4 fact be generalists at the species level.

5 Despite the fact that long-term, across-population studies can improve our  
6 understanding of the diverse selective pressures that drive floral evolution in zoogamous  
7 angiosperms (Brody, 1997; Aigner, 2005; Herrera *et al.*, 2006), most reports of intraspecific  
8 variation in pollination is biased towards specialized pollination systems, and information  
9 concerning spatial and temporal variation in pollination is scarce for generalist plant species  
10 (Herrera, 2005; Gómez *et al.*, 2009a; Gómez *et al.*, 2009b; Kuppler *et al.*, 2016).

11 One of the plant families often associated with generalist pollination systems is  
12 Apiaceae (Faegri and van der Pijl, 1966; Proctor *et al.*, 1996; Corbet, 2006; Zych *et al.*, 2007),  
13 a taxon characterised by a high degree of floral and inflorescence uniformity (Bell, 1971; Bell  
14 and Lindsey, 1978). Indeed, Olesen *et al.* (2007) estimated that several members of Apiaceae  
15 are amongst the “top 10” plant generalists, based on the number of insect taxa that visited  
16 their flowers. These plants included *Angelica sylvestris* L., which was visited by at least 245  
17 insect species occurring within the plant’s geographic range (Ellis and Ellis-Adam, 1993).  
18 This general statement, however, may not necessarily be true for all members of the Apiaceae,  
19 since more specialized plant-pollinator relationships have been described for other  
20 umbelliferous species (Lindsey, 1984; Zych, 2007; Niemirski and Zych, 2011; Cursach and  
21 Rita, 2012b; Zych *et al.*, 2014). These relationships may result from variation in many subtle,  
22 pollination-related characters that, in certain cases lead to “cryptic specialization” of  
23 pollination systems (Bell, 1971). Such characters include, for example, umbel density (Bell  
24 and Lindsey, 1978; Lindsey and Bell, 1985; Bisht *et al.*, 2008) and sex ratio (Pickering and  
25 Hill, 2002), the degree of dichogamy (Cruden and Hermann-Parker, 1977; Webb, 1981;

1 Schlessman and Barrie, 2004), nectar composition and secretion (Lindsey and Bell, 1985;  
 2 Stpiczyńska *et al.*, 2015) and floral scent profiles (Borg-Karlson *et al.*, 1994; Tollsten *et al.*,  
 3 1994; Tollsten and Øvstedal, 1994). Unfortunately, we are aware of only ten studies that  
 4 address spatial and/or temporal variation in the pollination of natural populations of this large  
 5 and economically important family (Lindsey and Bell, 1985; Kaye and Kirkland, 1994;  
 6 Lamborn and Ollerton, 2000; Pérez-Bañón *et al.*, 2007; Zych, 2007; Davila and Wardle,  
 7 2008; Danderson and Molano-Flores, 2010; Niemirski and Zych, 2011; Cursach and Rita,  
 8 2012a; Zych *et al.*, 2014), only five of which address both phenomena. The general picture  
 9 that arises from the aforementioned studies is intriguing. For example, *Daucus carota*  
 10 (Lamborn and Ollerton, 2000) and Australian *Trachymene incisa* (Davila and Wardle, 2008)  
 11 exhibited great fluctuations in pollinator assemblages. This contrasts markedly with data  
 12 obtained for the reputedly super-generalist *A. sylvestris*, which is visited by numerous insect  
 13 taxa, but pollinated only by a taxonomically relatively narrow group of muscoid and syrphid  
 14 flies (Niemirski and Zych, 2011). Furthermore, the composition of the pollinator assemblage  
 15 for *A. sylvestris*, like that of another umbellifer, namely *Heracleum sphondylium* (Zych,  
 16 2007), remained constant in subsequent years. Therefore, it would appear that, despite their  
 17 superficial uniformity, umbellifers display a whole range of different pollination strategies,  
 18 perhaps even including “cryptic specialization” (Bell, 1971), with certain, subtle floral  
 19 features attracting (or repelling) particular visitors to the flower. More data, however, relating  
 20 to both temporal and spatial variation in the pollination system, is required if we are to  
 21 investigate this hypothesis. To that end, we undertook assessment of the spatio-temporal  
 22 variation present in the generalist pollination system. For the above reasons, we selected, as a  
 23 model plant for this study, *Angelica sylvestris*. This species has a broad distribution range  
 24 (Cannon, 1968) and is among the very few European Apiaceae taxa that have been  
 25 extensively studied for both their pollination and floral biology under local conditions (Knuth,

1898; Zych *et al.*, 2007; Niemirski and Zych, 2011). Furthermore, in terms of its pollination system, this species is considered a “supergeneralist” (Ellis and Ellis-Adam, 1993; Olesen *et al.*, 2007), even though its key pollinators appear exclusively to be dipterans (Niemirski and Zych, 2011). Of particular interest were: (1) whether different populations of the species exhibited any variation in pollinator assemblages (also, over the course of several years) that could lead to local specialization of the pollination system, (2) whether such variation (if present) coincided with phenotypic variation (including nectar and scent characters), indicating the occurrence of co-evolutionary processes, and finally (3) whether it is adaptive, i.e., it resulted in increased reproductive output by populations.

## MATERIALS AND METHODS

### ***Angelica sylvestris* L.**

*Angelica sylvestris* L. (Wild Angelica) is a common component of the European flora, is distributed almost throughout the whole of Europe, and is usually found in wetlands, damp meadows and shady places (Cannon, 1968). It is a member of a large genus comprising ca. 110 species (Mabberley, 2008). This herbaceous perennial produces cauline leaves arranged in a rosette, and erect flower stems up to over 2 m tall (Cannon, 1968). *Angelica sylvestris* reproduces by seeds, and to date, no indication of vegetative reproduction has been reported. Small (ca. 2-3 mm in diameter), open flowers are arranged in large multi-layered inflorescences termed compound umbels (Fig. 1). Petals are greenish-white to pale pink in colour, and flower symmetry is mostly actinomorphic, but the outer flowers, arranged in umbellets, may be weakly zygomorphic. The flowers are dichogamous, and plants generally exhibit strong protandry at the level of the individual flower, the inflorescence and the whole plant. However, in some individuals, a short overlap in sexual phases is possible within any given umbel (Niemirski and Zych, 2011). Flowers are visited by insects for pollen and for

nectar which, as in many other Apiaceae, is produced in both flower sexual phases by the swollen base of the style – a structure called the stylopodium (Stpiczyńska *et al.*, 2015). As demonstrated by these authors, the nectar of *A. sylvestris* is hexose-rich and composed of sucrose, glucose and fructose, as well as small amount of amino acids. Nectar production is male-biased, being more than three-fold greater than in female phase flowers. This, however, does not appear to result in discrimination against pistillate phase by insect visitors (Niemirski and Zych, 2011), a phenomenon that has been recorded for certain other Apiaceae species (Schlessman *et al.*, 2004; Davila and Wardle, 2007; Zych, 2007). In terms of its pollination system *A. sylvestris* is regarded a supergeneralist (Olesen *et al.*, 2007) since its umbels are visited by a wide range of insects representing several taxonomic orders (Ellis and Ellis-Adam, 1993; Zych *et al.*, 2007; Niemirski and Zych, 2011). Although these flowers have no morphological adaptations that would restrict the access of insect visitors to floral rewards, recent analysis of insect effectiveness revealed that in NE Poland, they are chiefly pollinated by a narrow assemblage of muscid and syrphid flies (Niemirski and Zych, 2011).

## Study populations

For over three years (2011-2013), we conducted field observations and insect sampling for three Central European *A. sylvestris* populations located along an approx. 700 km SW-NE transect: (1) Milicz, Lower Silesia region, SW Poland, N 51°30'36'' E 17°18'23'', 132 m a.s.l., hereafter referred to as the SW population; (2) Kleczkowo, Mazovia region, NE Poland, N 53°02'33'' E 21°51'37'', 106 m a.s.l., hereafter referred to as the Central (C) population (the same population was earlier studied by Zych and co-workers; Zych *et al.*, 2007; Niemirski and Zych, 2011); and (3) Šiauliai, Šiauliai region, N Lithuania, N 55°47'52'' E 23°18'27', 73 m a.s.l., hereafter referred to as the NE population. All three populations grew in similar wet meadow ecosystems within a mosaic of open and forest landscapes.

## **Insect visitors to natural populations**

Field observations of insects were completed during the periods July and August 2011-2013, which is the peak flowering time for *A. sylvestris* in these regions. For insect observations and sampling, a slightly modified method to that described by Niemirski and Zych (2011) was employed. In 2011, for each population, we completed 12 rounds of observations of both umbel sexual phases (6 for female phase umbels and 6 for male phase umbels), and during 2012-13, 24 rounds annually were completed (12 for female phase umbels and 12 for male phase umbels). Each round lasted consisted of three phases: random selection of umbel, video recording (15 min, using digital camera HDRXR106, Sony Corp. Japan) and insect sampling (15 min, using an entomological net or directly into plastic vials) , totalling 90 h of observations and insect sampling over three years. Once selected, umbels were not excluded from the subsequent round, and therefore it is possible that the same umbel was observed more than once.

For each study day, observations commenced at 1000 h and ended at 1600 h, at the latest. No more than four rounds for a particular umbel sexual phase were completed in a single day, which means that for a single populations, observations lasted for at least three full days or, more usually, longer during inclement weather (strong winds or rain), observations were halted and re-commenced on subsequent days at the appropriate hour until all planned rounds were completed for any given umbel sexual phase per given year and population. Only primary umbels in either the male or the female phase were chosen for observations, since, in most umbellifers, e.g. in the genus *Angelica*, these are mainly responsible for seed production (Ojala, 1986).

During insect sampling all individuals visiting the selected umbel were collected, killed with ethyl acetate, and pinned and stored for further investigation of their body pollen

loads. We excluded from the analyses aphids and other small, sap sucking insects (e.g. Thysanoptera), together with insects smaller than 1 mm, as these animals were usually observed clinging to the stylopodium and, even when moving around the flower, were too small to make effective contact with the stigma or anthers. Despite recent suggestions that ants may pollinate some umbelliferous species (Carvalho *et al.*, 2008; Cursach and Rita, 2012a), we also excluded Formicidae from our analysis, since their ineffectiveness as pollinators was recently confirmed for a similar system (Zych *et al.*, 2014).

The video recordings were analysed in the laboratory for the number of visits to individual inflorescences and the proportion of umbellets visited by a single insect within a particular compound umbel. As with other studies involving umbellifers, (e.g. Lamborn and Ollerton, 2000; Niemirski and Zych, 2011; Zych *et al.*, 2014), we grouped insect visitors on taxonomical grounds into the following visitor morphogroups: wasps (predatory wasps of the family Vespidae), hoverflies (insects of the family Syrphidae), muscoid flies (large >5 mm insects of the families Calliphoridae, Muscidae, Sarcophagidae, Tachinidae), beetles (insects of the order Coleoptera) and bees (Apoidea). Rare visitors from other taxonomic groups (e.g. butterflies, small flies <5 mm etc.) were pooled as ‘Other’.

### **Insect body pollen loads**

For the preparation and analysis of insect body pollen loads, the gelatin-fuchsin method of Dafni *et al.* (2005) was used. Using fine forceps, a Nikon SMZ 645 stereomicroscope and a small cube (ca. 3-4 mm<sup>3</sup>) of gelatin-fuchsin jelly, all visible pollen grains adhering to the insect body surface were removed. The jelly was then transferred to a glass microscope slide, a coverslip applied, and the slide gently heated over a flame to make a semi-permanent preparation. A Nikon Eclipse 100 light microscope was used to score the total number of pollen grains of both *A. sylvestris* and non-*A. sylvestris* taxa (hereafter referred to as ‘other’

pollen grains). The loads were sub-sampled (all pollen grains were scored for nine areas evenly distributed over the cover slip) and the results, after calculating the arithmetic mean of each count, were extrapolated to the area of the coverslip to obtain the pollen load in any given sample. This method gives results comparable to counting total body pollen loads (Zych, 2007).

### **Pollinator importance**

For estimating the pollination effectiveness of insect visitors, we adopted the approach used by Niemirski and Zych (2011), namely, an indirect method (pollinator importance measure, *I*) based on counts of insect pollen loads, observations of insect frequency, and their abundance and behaviour on the flowers:

$$I_x = V \times U \times PL,$$

where:  $I_x$  – importance of insect species X,  $V$  – abundance (no. of recorded visits of species X + no. of captured individuals of species X)/(total no. of recorded visits + total no. of captured individuals),  $U$  – umbel penetration ratio (mean no. of umbellets visited by species X within an umbel / mean no. of umbellets in an average umbel in the population surveyed),  $PL$  – average pollen load (number of pollen grains) carried by an individual of species X.

*I* was calculated separately for each population and for every study year, and then totalled for all the insect groups so as to obtain the maximum possible value. The importance coefficient (IC) of each insect group was expressed as a percentage of the total value.

### **Floral phenotypes and population reproductive output**

In order to assess differences in floral characters and relative plant reproductive success in each population at the end of the growing season (September), we randomly collected 20-25 whole individual plants and mounted them on herbarium sheets. Later in the lab, using

1 stereoscopic binoculars, for each plant we counted , for each plant, the number of umbellets in  
2 primary (main) umbels (equivalent to the size of an inflorescence) and assessed the number of  
3 male/bisexual flowers and fruit production. For the latter, we used the pooled counts from  
4 three randomly chosen umbellets per main umbel. The number of bisexual flowers was  
5 calculated as the sum of fruiting and non-pollinated flowers (ones that failed to form fruit).  
6 Seed-set was calculated as the number of seeds divided by the number of bisexual flowers.  
7 We focused on inflorescence features since, in the Apiaceae, umbels rather than the minute  
8 flowers are recognized as units of pollination (Bell and Lindsey, 1978). Additionally, in 2012,  
9 we collected five fully ripe seeds from the main (first order) umbel of each plant and weighed  
10 them using an analytical balance (AS 60/220/C/2 RADWAG, Radom, Poland).

## 12 **Nectar sampling**

13 To avoid intra-population variation in nectar production caused by environmental conditions,  
14 e.g. soil fertility, water stress etc., we used a common-garden approach for nectar sampling. In  
15 early spring 2012 and 2013, for each study population, we randomly collected 25 young plant  
16 rosettes and transplanted them into individual pots using standard garden soil. These were  
17 kept under ordinary climatic conditions in the Botanic Garden (mean annual temperature  
18 +8.3°C and mean annual rainfall 550.6 mm; based on data for years 1951-2010) until the  
19 flowering stems appeared. Each season, before flowering commenced, plants for nectar  
20 experiments were transferred to a closed greenhouse chamber to prevent visits by insects, and  
21 following flowering, were again transferred to the common-garden field. Throughout the  
22 whole growing season the plants were copiously watered.

23 Samples of nectar were collected in 2012 and 2013 from 15-25 flowers at the male  
24 stage and, owing to the smaller volume of available nectar, from 30-40 flowers at the female  
25 stage. The small volume of nectar produced meant that we were only able to collect 23

1 samples in 2012 (9, 6 and 8, respectively from SW, C, and NE plants) and 39 in 2013 (13  
2 samples for each population). The nectar was subsequently expelled from the pipette onto a  
3 refractometer prism RL-4 (PZO, Warszawa, Poland) and nectar sugar concentration  
4 calculated and expressed as percentage weight of nectar.

5 In order to determine the composition of nectar sugars during both floral sexual stages,  
6 nectar from fifty flowers for each stage of development was collected using micro-pipettes  
7 and analysed by isocratic HPLC in conjunction with LC1 Waters system. A 20 µl aliquot of  
8 both sample and standard solution was injected. Water (MilliQ, pH 7), with a flow rate of 0.5  
9 ml/min, was used as the mobile phase. Sugars were separated in a Waters Sugar-Pack I  
10 column (6.5-300 mm) maintained at 90°C, and identified by a refractive index detector  
11 (Waters 2410). The content of fructose, glucose and sucrose contents were determined and  
12 expressed as the percentage of total sugars.

13 Amino acid analysis of a 10 µl sample of nectar collected from 50 flowers was  
14 performed by gradient HPLC using an ion exchange Novapak C18 (15 mm × 4.6 mm)  
15 cartridge, with guard column maintained at 37°C, and a Waters 470 scanning fluorescence  
16 detector (excitation at 295 nm, detection at 350 nm). A solvent composed of TEA-phosphate  
17 buffer (pH 5.0) mixed with a 6:4 acetonitrile-water solution was used as the mobile phase at a  
18 flow rate of 1.0 ml/min. According to AccQtag protocol (Waters Corp.), the selected volume  
19 of each reconstituted sample was amino acid-derived (Cohen and Micheaud, 1993) with AQC  
20 fluorescent reagent and 0.02 M borate buffer (pH 8.6). In addition to all the protein amino  
21 acids, standards of β-alanine, citrulline, L-homoserine, α-aminobutyric acid (AABA), γ-  
22 aminobutyric acid (GABA), hydroxyproline, ornithine and taurine were also used.

## 24 Scent sampling

1 The same common-garden approach was used for scent sampling in 2016. Dynamic  
2 headspace scent samples were collected from one inflorescence in full bloom per plant  
3 following the method described by Kuppler *et al.* (2016). Potted plants from each population  
4 were transferred to the laboratory where samples of floral scent were collected from unpicked  
5 umbels. The umbels were enclosed within a polyester oven bag (Toppits®, Germany) for 15  
6 min and the emitted volatiles were then trapped on 1.5 mg Tenax (mesh 60– 80; Supelco,  
7 Bellefonte, PA, USA) and 1.5 mg Carbotrap B (mesh 20– 40, Supelco) in a quartz vial  
8 (Varian Inc.; length 15 mm, inner diameter 2 mm) for 2 min using a membrane pump (G12/01  
9 EB, ASF Rietschle-Thomas, Puchheim, Germany) with a flow rate of 200 ml min<sup>-1</sup>. All  
10 samples were collected between 10:00 and 14:00 h. Scent samples were analysed using an  
11 automatic thermal desorption system (TD-20, Shimadzu, Japan) coupled with a GC–MS  
12 (model QP2010 Ultra EI, Shimadzu, Japan). The GC-MS was equipped with a ZB-5 fused  
13 silica column (5% phenyl polysiloxane; 60 m long, inner diameter 0.25 mm, film thickness,  
14 0.25 µm, Phenomenex) and the column flow (carrier gas: helium) was set to 1.5 ml/min. The  
15 GC oven temperature started at 40°C (split ratio 1:1), then increased by 6°C per minute to  
16 250°C and held constant for 1 minute. The MS interface worked at 250°C. Mass spectra were  
17 taken at 70 eV (in EI mode) from m/z 30 to 350. The GC/MS data were processed using the  
18 GCMSolution package (Version 2.72, Shimadzu Corporation). Compounds were identified by  
19 comparison of mass spectra and retention times with standard compounds, which are  
20 commercially available. Alternatively, compounds were identified using the mass spectral  
21 libraries Wiley 9, Nist 2011, FFNSC 2, Essential oils and Adams 2007, as well as the  
22 database available in MassFinder 3. The compounds found in the flowers were compared with  
23 those present in the blanks (empty oven bags) so as to determine which compounds were  
24 specifically emitted by flowers.

## **Transplantation experiment**

In order to check the performance of plants from various sources in native vs. non-native pollinator environments in 2015 and 2016, for each study site (SW, C and NE), we created a mixed population composed of potted plants originating from all three natural sites. Like the protocol used for nectar sampling, each year, plants for potting were collected from source populations in spring, potted in standard garden soil and kept under prevailing weather conditions in the botanic garden until flowering. The plants were then transported to source populations so as to create experimental populations consisting of 24 plants in total (8 plants, respectively, from M, S and K). Pots with plants were arranged according to the scheme shown in Fig. A under ‘Supplementary Materials’. During flowering, insect activity on the primary umbel of each plant was recorded using digital video cameras. This was performed twice for each plant (in male and female phase), and video recordings lasted 5 min each. Video recordings were analyzed in the lab for insect visits (and as previously, insect visitors were assigned to six morphogroups: bees, wasps, beetles, flies, syrphids and ‘other’). Experimental plants were left in the field until the early stage of fruit-ripening and later transported to the botanic garden, where the number of fruits, non-pollinated female flowers and male flowers in primary umbels were scored. Our hypothesis stated that plants should perform better, both in terms of insect visitation frequency and resulting seed-set in their populations of origin.

## **Statistics**

Statistica 13.1 (Dell Inc.) was used for most statistical calculations. For comparing most reproductive characters between populations (except for seed-set) we used one-way ANOVA. In order to account for natural variation between study years and the resultant errors that could affect the model (Bolker *et al.*, 2009), for visit frequency and nectar production, we

used the mixed-model ANOVA approach, treating the study year as a random factor. Where appropriate, the data were square-root-transformed to obtain normal distribution. Data on fruit-set and nectar concentration could not be successfully transformed, and therefore nonparametric Kruskal-Wallis ANOVA was used for comparisons.

To test whether nectar amino acid and floral scent composition differ between populations, we used ‘random forest’ analysis (Breiman, 2001) implemented in the R package randomForest (R Development Core Team, 2011). This machine-learning algorithm allowed to assign plant individuals from the three study populations to pre-defined groups (SW, C, NE) and to estimate the importance of particular amino acids and scent compounds for correctness of the assignment. This classification tool has been shown to be very powerful in classifying samples characterized by multiple variables (Junker and Keller, 2015). For our analysis, ntree = 10.000 bootstrap samples were drawn with  $mtry \sim \sqrt{\text{variables}}$  randomly selected at each node. Random forest returns a confusion matrix that shows the number of correctly assigned samples for each population (either SW, C or NE), the proportional class error and a variable importance E for each amino acid (AA) and scent compound. A high variable importance indicates that this amino acid or scent component strongly separates the populations.

## RESULTS

### Insect visits and behaviour

During the course of three years, we recorded 8477 insect visits to our study plants. The overall visit frequency was  $32 \pm 25$  visits per census (15 min), and this did not significantly differ between populations (data pooled over three study years; mixed-model ANOVA on square-root-transformed data, with population as fixed factor and year as random factor,  $F_{2,168} = 2.927$ ,  $p=0.06$ ).

All visitor morphogroups were present for each site and, year by year, most insect visits (70-91%) were made by dipterans (muscoid flies and Syrphidae) and beetles. The relative contribution of these insects, however, was highly variable between populations and quite constant within each population (Fig. 2). Beetles were the main visitors to the SW population (depending on the year, 48-64% of all recorded visits), whereas in the C population, they represented only 1-10% of visits, and for all study years this population was dominated by dipterans (depending on the year, 60-90% of all recorded visits). The pattern of visits for the NE population was less consistent. In 2011, 67% of visits was made by beetles, whereas Diptera were predominant in 2012 (62%) and 2013 (80%). In 2012, we recorded increased visits to all sites by wasps (9%, 4% and 4%, respectively for SW, C and NE population), whereas in 2013, the warmest of all study years, a considerable proportion of visits was made by bees, which are usually rare (13%, 28% and 5%, respectively for SW, C and NE population; in years 2011-2012 for all populations  $\leq 5\%$ ).

Insect behaviour on umbels (calculated as umbel penetration ratio, i.e. the proportion of visited umbellets in an umbel) was variable across both years and populations, and showed no particular pattern [Fig. B, **Supplementary Information**] (since the data on insect behaviour on umbels was to be used to compare visitation patterns of particular insect guilds, we employed nonparametric Kruskal-Wallis ANOVA). For example, for populations SW and NE we detected significant differences in insect activity each year, whereas for population C they were significant only for 2012.

### **Insect body pollen loads**

We analysed 2741 insect body pollen loads (987, 1020 and 734, respectively for SW, C and NE population) and, above all, found large variation in pollen loads between individual members of a particular morphogroup (averages for all recognised morphogroups for three

populations over three study years are presented in [Supplementary Information]). Both the number of *A. sylvestris* and ‘other’ pollen grains carried by an individual insect varied over four to five orders of magnitude, from virtually none to the largest pollen load of 541,227 *A. sylvestris* grains estimated for a wasp captured in 2011 on male phase umbels in SW population. BY comparison, the largest ‘other’ pollen load of 81,037 grains found on a beetle netted in 2011 on a female phase umbel in the C population. Most of the analysed loads (approx. 66%) were composed of both types of pollen, and the quantity of *A. sylvestris* and ‘other’ pollen was positively correlated (Pearson’s  $r^2=0.1896$ ,  $p<0.005$ ). Fifteen percent of all captured insects carried no pollen at all, whereas 15% and 4% of loads were composed of only *A. sylvestris* or ‘other’ pollen grains, respectively. Overall, both the largest *A. sylvestris* load and largest ‘other’ pollen load were carried by seldom-observed wasps (respectively,  $22,801\pm76,655$  and  $3804\pm9610$  pollen grains, mean and SD). In the case of *A. sylvestris* pollen, the results for wasps exceeded those for other visitor groups by an order of magnitude. In population SW, these insects were the only visitor guild that significantly differed from others in their mean body pollen loads ( $p<0.001$ , post-hoc Tukey’s HSD test for uneven N), data pooled for study years and umbel sexual phases). The remaining guilds bore average pollen loads of equal size (Fig. 3).

### **Pollinator importance**

For all study populations, during the course of three years of sampling, the most important pollinators were flies (muscid and syrphids), beetles and hymenopterans (bees and wasps), with very marginal contribution from other insect groups. Interestingly, similarly like the visitation data, the relative contribution of particular insect groups remained highly variable between populations, but relatively constant across years (Fig. 4). Generally, flies were the key pollinators of plants in C and NE populations (IC range 45-96%), whereas beetles

1 contributed most to the pollination of SW plants, but played rather marginal role in the  
2 pollination of the two remaining populations (IC from 0 to a maximum of 17% in 2012 for  
3 NE). The results for hymenopterans (bees and wasps) were more variable across years, and  
4 the morphogroups usually replaced each other, i.e. if the contribution of bees was significant  
5 (as in 2013 for SW or C), wasps were effectively absent and *vice versa* (2011 in SW or NE,  
6 and 2012 in C).

### 8 **Floral characters and reproductive success of populations**

9 We did not detect differences in umbel sex ratios: in plants from all three populations, main  
10 (primary) umbels were composed only of bisexual flowers. Our study populations differed in  
11 primary umbel size (measured as the mean number of umbellets), but this was inconsistent  
12 over the years. In 2012, the largest primary umbels were produced by plants from NE and SW  
13 populations, whereas the smallest were found in plants from the C population. By contrast, the  
14 latter were the largest in 2013 (owing to unexpectedly late mowing of the NE population that  
15 year, we were unable to collect plants from this site). In either year, all populations scored  
16 nearly 100% fruit-set, and we found no significant differences between study sites. We did,  
17 however, find differences in seed mass, with significantly heavier seeds recorded for SW  
18 population (Table 1).

### 20 **Nectar**

21 Plants from study populations produced hexose-rich nectar composed of fructose, glucose and  
22 sucrose (sucrose/[glucose + fructose] ratio of 0.18, 0.19 and 0.12, respectively for SW, C and  
23 NE plants), with similar proportions for the three detected sugars (Fig. C; supplementary  
24 materials).

In 2012, plants from different populations produced nectar of similar sugar concentration (overall, the 2012 mean was  $26.2 \pm 14.4\%$ ; since in *A. sylvestris*, sugar concentration was shown to be similar for both floral sexual phases (Stpiczyńska *et al.*, 2015), we pooled data from male and female phase umbels; Kruskal-Wallis ANOVA<sub>2012</sub>:  $H(2, N=23) = 3.450345$   $p = 0.178$ ), however, in 2013, they differed in that respect (Kruskal-Wallis ANOVA<sub>2013</sub>:  $H(2, N=39) = 9.150101$   $p = 0.010$ ). The lowest sugar concentration for 2013 ( $15.4 \pm 5.5\%$ ; mean and SD) was found in C plants, but was significantly higher,  $23.1 \pm 6.4\%$ , for SW plants (sugar nectar concentration for NE plants,  $20.9 \pm 6.5\%$ , did not differ significantly from that of either group).

SW and C plants produced nectar that was relatively richer in amino acids (respectively,  $0.12 \pm 0.07$  and  $0.16 \pm 0.08$   $\mu\text{mol/ml}$ ), whereas the amino acid concentration for the NE plants was approx. three-fold lower ( $0.05 \pm 0.01$   $\mu\text{mol/ml}$ ). Unfortunately, due to low nectar volumes, we were able only to analyze two male phase samples per population, and the recorded differences were not statistically significant (Kruskal-Wallis ANOVA  $H_{(2, N=6)} = 3.7143$   $p = 0.16$ ).

We detected 19 different amino acids (AAs) in the collected samples of nectar (Fig 5). Only ALA, PRO, PHE, BALA and BABA were detected in at least one sample from each of the three populations, and many AAs were population-specific. For example, ASN, GLN, CIT, ARG, GLY TAU, VAL and ILE were recorded only for the C population, whereas LYS was unique for NE, and HYS for SW samples. Random forest analysis assigned all SW samples to NE population, indicating that those two are not well separated and that C plants differed in nectar AA profiles from individuals comprising the other two populations. The estimated error rate for the whole data set was 66.67%, indicating that 2/3 of samples were not correctly assigned to the populations. Unfortunately, low number of samples obtained limits further inference.

## Floral scent emissions

In total, we detected 44 floral scent compounds in 34 samples collected from plants derived from three study populations. Our populations overlapped for the presence/absence of most compounds (see Table. 1 in supplementary material), but the proportional composition differed between populations (random forest out of basket estimate of error rate: 32.35 %). Ten out of 13 samples from the NE population were correctly assigned to this population (random forest class error: 23.08 %). Assignments of samples from C and SW populations received slightly higher random forest class errors, namely, 37.5 % and 38.46%, respectively. This result is also reflected in the ordination (non-metric multidimensional scaling NMDS based on Bray-Curtis distances of quantitative scent emissions, Fig. 6). Despite some overlap of the samples from different populations, the population centroids were clearly separate (population factor fitted onto NMDS:  $r^2 = 0.15$ ,  $p = 0.033$ , R package *vegan* (Dixon, 2003)).

## Transplantation experiment

Even before flowering, some of our experimental plants were attacked by powdery mildew. Furthermore, developing fruit often attracted sap-feeding insects (hemipterans). Consequently, some inflorescences died before flowering could occur, which in some cases drastically reduced our sample size for insect video recordings and seed-set analysis.

Generally, regardless of population, all plants were visited equally by insects (Fig. 7). The only exception was recorded in 2015, when in C and NE populations, C plants were visited more frequently than NE plants (Kruskal-Wallis ANOVAs, respectively, for C-population  $H_{(2, N=46)} = 6.8110$   $p = 0.03$  and NE-population  $H_{(1, N=34)} = 10.2235$   $p = 0.001$ ; unfortunately, that year we lost all SW plants in the NE population due to powdery mildew).

Seed-set for our experimental (potted) plants was lower than data obtained from naturally occurring individuals (69-97%), but for both years, it remained constant within source populations, regardless of plant origin (Table 2).

## DISCUSSION

Our study documented substantial geographic and low temporal variation in the pollination system of *A. sylvestris*. Population SW was visited and pollinated mainly by beetles, whereas the remaining two (C and NE), chiefly by dipterans. Although, qualitatively, visitor assemblages remained similar, i.e. all main morphogroups were present in all three study populations, the contribution of a given pollinator morphogroup remained relatively constant over the years. Despite increased beetle visitations in the NE population (year 2011), flies remained the key pollinators. Furthermore, the results for C population agree with those obtained previously for the same locality by Niemirski and Zych (2011), who recorded that over two study years (2006 and 2007), dipterans were consistently responsible for approx. 90% of pollination events. This suggests that the temporal consistency of pollinators in the case of our study species is maintained over long periods, a necessary precondition for the local specialization of populations depended on pollination by flies and beetles. However, for specialization to occur, it is necessary that the observed groups of insect visitors differ in their capacity to pollinate (Gómez and Zamora, 2006). According to the latter authors, this can be achieved by variations in the abundance and effectiveness on a flower, together with preferences towards, or mechanical compatibility with some floral phenotypes. As a result, different pollinator groups are able to affect plant fitness to varying degrees. Therefore, specialization is unlikely when different pollen vectors play the same role as selective agents due to their functional equivalency (Gómez and Zamora, 1999; Zamora, 2000). This appears to be the case in *A. sylvestris*, where most visitor groups, including dominant beetles and flies,

1 seem to be equally effective on flowers. For example, we observed no difference in body  
2 pollen loads between pollinator morphogroups. Similar spatial (and temporal) turnover of  
3 equivalent pollinators can perhaps be observed for some other umbellifers, such as *D. carota*,  
4 because various populations of this species in the UK are mainly visited by beetles  
5 (*Rhagonycha fulva*; which was also abundant in our study), sawfly (*Thenthredo* sp.), or  
6 dipterans carrying similar amounts of pollen (Lamborn and Ollerton, 2000). According to  
7 these authors, the plants rely on functionally similar groups that fluctuate over the years in  
8 terms of their abundance, but collectively do not differ in their importance.

9         The only marked exception in our study were wasps whose body pollen loads, in the  
10 SW population exceeded those of other pollinators by one order of magnitude. This result is  
11 probably due to the fact that, of the recorded insect visitors, Vespidae had the largest bodies.  
12 These insects, however, were also quite erratic visitors, both in SW and the other two  
13 populations, and were almost completely absent from all three study sites in 2013. This  
14 finding resembles that for a three year-long study of umbelliferous *Ostericum palustre*, where  
15 wasps were key pollinators for a single season, and virtually absent for the rest (Zych *et al.*,  
16 2014). Generally, wasps seem to be rather opportunistic visitors to Apiaceae flowers (Zych,  
17 2002; Zych *et al.*, 2014), but their presence and sometimes aggressive behaviour can affect  
18 the performance of other pollinators. Fluctuations in the annual abundance of wasps may be  
19 caused by e.g. unfavourable climatic conditions (Archer, 2001). They are thus unlikely to  
20 exert a significant selective pressure on the studied system. Nevertheless, where other visitor  
21 groups are concerned, even the smaller average pollen loads were more than sufficient to  
22 pollinate *A. sylvestris* flowers, which contain only two ovules. Indeed, seed-set was invariably  
23 close to 100% in all studied localities, and the populations did not seem to be pollen-limited.  
24 In such situations, if plants bear few ovules per flower and thus require relatively few pollen  
25 grains for a full seed-set, the probability that two visitors are equally effective pollinators

increases (Johnson *et al.*, 1995). This, contrary to the earlier suggestions of Niemirski and Zych (2011), minimizes the probability that *A. sylvestris* is specialized for pollination by the most abundant insect visitors. In most cases, for example, we found no significant correlation between the per-visit effectiveness of each visitor morphogroup (measured here as body pollen loads) and their abundance, which, according to some authors (Gómez and Zamora, 2006; and references therein) indirectly indicate the absence of specialization. This lack of specialization on the most abundant visitors was further confirmed by our transplantation experiment. Although seed-set in potted plants was smaller than in natural populations, and was probably caused by suboptimal growing conditions, we observed neither differences in insect activity, nor in the seed-set of plants derived from natural populations grown in common-garden conditions. One marked exception was a reduction in insect visits to NE plants in C and NE populations, in 2015, which, however, did not result in decreased seed-set. In view of this, our findings that differences in insect visitor assemblages were generally not associated with changes in the floral characters of populations seem to confirm the very generalist pollination strategy of the investigated species. This similarity also extends to floral rewards, especially nectar characteristics, which were generally constant across our study sites, at least as far as sugars concentration and profile were concerned. The observed sugar profile was also similar to that recorded in previous reports for *A. sylvestris* (Stpiczyńska *et al.*, 2015), thus confirming proposals concerning the conservative character of this floral trait within a species (Roy *et al.*, 2017). The most noticeable exception was overall nectar amino acid (AA) composition of C population whose nectar, unlike that of the other two populations, contained several AAs, including non-protein AAs, such as citrulline or taurine, absent elsewhere. This variation between populations challenges previous ideas regarding the species-specific constancy of nectar AAs (Baker and Baker, 1986), but affirms more recent studies which demonstrate considerable variation in this nectar trait (e.g. (Lanza *et al.*, 1995;

1 Terrab *et al.*, 2007; Gijbels *et al.*, 2014). Nevertheless, like a previous study of *A. sylvestris*  
2 nectar (Stpiczyńska *et al.*, 2015), our survey showed that proline and alanine are core  
3 constituents of the nectar AA profile in all surveyed populations, but also confirmed that the  
4 nectar contains a substantial proportion of non-protein  $\beta$ -alanine, which in the SW population  
5 comprised over 40% of all nectar AAs. Alanine and proline are generally common nectar AAs  
6 (Baker, 1977), the latter being preferred by many pollinators, especially bees, probably  
7 because of its role in insect flight (Teulier *et al.*, 2016). Non-protein AAs, such as  $\beta$ -alanine,  
8 may also contribute to the modification of the foraging behaviour of insects by bringing about  
9 changes to the regulation of their nervous systems (Nepi, 2014). Generally, insects seem to  
10 prefer high concentration of AAs, perhaps because of their alimentary value and specific taste  
11 (Baker, 1977; González-Teuber and Heil, 2009; Roy *et al.*, 2017), which might explain fewer  
12 visits to our experimental NE plants, which contained smaller quantities of AAs. Differences  
13 in AA composition, however, may also be related to factors other than pollinator attraction,  
14 for example, they may play a role in defence against fungi and bacteria (Nepi, 2014). In  
15 separated populations, they can also be correlated to variations in environmental traits such as  
16 precipitation, temperature and sunlight (Terrab *et al.*, 2007; Gijbels *et al.*, 2014), which might  
17 not have been revealed using our common-garden approach. This aspect of *A. sylvestris*  
18 pollination biology most certainly deserves further attention.

19       Regarding scent bouquets, our study plants produced floral odours that were mostly  
20 composed of various terpenoids and aromatic compounds, and resembled those found in  
21 earlier studies focused on *A. sylvestris* or other Apiaceae (Borg-Karlson *et al.*, 1994; Tollsten  
22 *et al.*, 1994). Such compounds are usually interpreted not as species-specific cues, but rather  
23 as a signal for a wide spectrum of insect visitors (Willmer, 2011). All volatile organic  
24 compounds found in *A. sylvestris* scent are common in floral bouquets. For example,  
25 benzaldehyde is found in 64% and phenylethanol in 54% of plant families investigated so far

(Knudsen *et al.*, 2006), nevertheless, their specific role may change depending on the insect taxon involved in the interaction (Junker, 2016). Although we found a clear qualitative overlap in scent bouquets between populations, the overall composition varied, the most prominent differences being related to 2-hydroxybenzaldehyde, benzaldehyde,  $\beta$ -myrcene,  $\beta$ -phellandrene and phenylethanol. Differences in quantitative scent composition were observed previously for other Apiaceae, e.g. *Laserpitium latifolium* (Borg-Karlson *et al.*, 1994), and perhaps these can also be related to non-pollinating insect visitors. Members of Apiaceae, for example, play host to numerous insect herbivores, with some relationships being very specialized (see e.g. Berenbaum, 1981), and it is known that floral scents serve both as signals to mutualists, and attractants or repellents to antagonists (Junker and Parachnowitsch, 2015; Junker, 2016). The latter can also modify floral fragrances, as reported for umbelliferous *Pastinaca sativa* (Zangerl and Berenbaum, 2009), suggesting that in our system also, some variation may be attributable to plant-herbivore interaction, rather than to pollinator attraction. This, however, requires further experimental study.

The overall picture appears to be the one of “adaptive wandering”, as described by Wilson and Thomson (1996 ). In this process, populations separated geographically by distance may diverge in response to local pollinator communities. In *A. sylvestris*, this, however, does not result in pollinator shift because the selective pressure acts too briefly to cause any substantial morphological and phenotypic changes that could exclude any type of pollinator. Furthermore, given the equivalency of *A. sylvestris* pollinators and their generalist character, any specialization could perhaps be only achieved by altering floral morphology, rather than traits like scent or nectar composition. An example of such a pathway was described for North American *Thaspium* and *Zizia* populations pollinated by the oligolectic bee *Andrena ziziae* (Lindsey, 1984). Despite the general attraction of various insect pollinators to these plants, the latter were regularly pollen-limited (seed set 50-80%), and their

1 adaptations for enhancing successful pollination included floral attractants (such as pollen and  
2 nectar available to the oligolectic bee) and subtle modifications of floral traits (such as corolla  
3 “tube” formed by folding of the petals and stamens; Lindsey and Bell, 1985).

4 Despite the above examples, umbellifers, especially *A. sylvestris*, appear to be  
5 morphologically well adapted to ecological generalization (Corbet, 2006). Specialization in  
6 this plant family, perhaps, occurs only under very special circumstances, for example, when  
7 the pollinator community is composed of insect visitor groups that clearly differ in their  
8 capacity to pollinate. However, despite the constancy of selective pressure over the years, the  
9 barrier to evolve any morphological improvements resulting in the fine-tuning of the flower  
10 towards particular pollinator types may well be the same as those architectural constraints on  
11 the floral bauplan that make umbellifers so uniform in their floral displays, and so successful  
12 in attracting large numbers of pollinators.

#### 14 **AUTHORS’ CONTRIBUTIONS**

15 MZ conceived the study; MZ, KR, and BS collected and assembled field data; MS and MN  
16 performed nectar analysis; KR, RRJ and MZ performed scent analysis; MZ and KR  
17 conducted transplantation experiments; MZ, RRJ and KR analyzed the data; MZ wrote the  
18 draft version of the paper. All authors contributed to the final version.

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## Figure legends

Fig. 1A-B. (A). Flowering shoot of *Angelica sylvestris* showing main (primary) umbel in fruit and flowering lateral (secondary) umbels. (B). Insect visitors to female (pistillate) phase umbel include *Rhagonycha fulva* beetles and muscid and calliphorid flies.

Fig. 2. Insect visits to umbels of *Angelica sylvestris* for years 2011-2013 in three study populations SW, C and NE, expressed as the percentage of total visits for a given year within a particular population; data based on captures and video records. Musc = Muscoid flies, Syrph = Syrphidae.

Fig. 3. Average body pollen loads carried by members of a particular pollinator morphogroup (error bars indicate 95% confidence limits of the mean). Data were pooled for study years and umbel sexual phases. Note the gap in the Y axis.

Fig. 4. Relative Pollination Importance (IC) of insect visitors to umbels of *Angelica sylvestris* in years 2011-2013 for three study populations: SW (Milicz, SW Poland), C (Kleczkowo, NE Poland) and NE (Šiauliai, NE Lithuania), based on visitation data, pollen loads and behaviour on flowers. Musc = Muscoid flies, Syrph = Syrphidae.

Fig. 5. Amino acid profile of *Angelica sylvestris* nectar produced by plants originating from three study populations SW, C and NE shown as the mean relative proportion of a particular compound in each population sample.

Fig. 6. Differences in scent composition of *Angelica sylvestris* flowers from three populations. Ordination shows results of NMDS based on Bray-Curtis distances on quantitative emission rates of flowers. Each sample is displayed as a circle, the population centroids are shown as triangles. Volatile organic compounds that significantly ( $p < 0.01$ ) correlate with samples in plot are shown as vectors. Emission rates of compounds shown in the plot (arrows) are greater in those samples that are plotted in the direction of the vectors.

Fig. 7. Average frequency (and SD) of total insect visits to experimental *Angelica sylvestris* plants in various population settings during the transplantation experiment over the course of two years. Means with various letters are different at  $p < 0.05$  (Kruskal-Wallis ANOVA calculated for particular site and study year).

## Supplementary Figures' legends

Fig. A. Arrangement of potted plants during transplantation experiments. The same scheme was used in each of the investigated populations.

Fig. B. Inflorescence penetration ratio (= proportion of visited umbellets in an umbel) by various visitor morphogroups for three study populations over three years of study. Results of Kruskal-Wallis ANOVA are shown. Error bars indicate standard deviation of the means. Vesp = wasps, Bee = bees, Syrph = hoverflies, Musc = Muscoid flies, Col = beetles.

Fig. C. *Angelica sylvestris* nectar sugar composition in the surveyed populations, expressed as a relative % of Fructose (Fru), Glucose (Glu) and Sucrose (Suc) on total sugars.

**Table 1.** Reproductive characters of plants from study populations; measurements were done on dry specimens. Data are given for primary (main) umbels, and presented as means  $\pm$ SD (sample size). Means with various associated letters are different at  $p < 0.05$ , post-hoc Tukey HSD test for different N. Data on fruit-set could not be successfully transformed, and therefore, nonparametric Kruskal-Wallis ANOVA was used for comparisons. In 2013, owing to unexpectedly late mowing of the study site, we were unable to collect plants from the NE (Šiauliai) population.

	SW (Milicz)	C (Kleczkowo)	NE (Šiauliai)	p
inflorescence size 2012	30 $\pm$ 7 (28) <sup>a</sup>	25 $\pm$ 7 (27) <sup>b</sup>	36 $\pm$ 8 (12) <sup>a</sup>	<0.001
inflorescence size 2013	22 $\pm$ 5 (29)	30 $\pm$ 7 (14)		<0.001
mean fruit-set 2012	0.99 $\pm$ 0.02 (24)	0.95 $\pm$ 0.14 (27)	0.95 $\pm$ 0.14 (12)	ns
mean fruit-set 2013	0.95 $\pm$ 0.10 (29)	0.98 $\pm$ 0.06 (14)		ns
mean seed mass [mg]	2.1 $\pm$ 0.6 (175) <sup>a</sup>	1.8 $\pm$ 0.6 (125) <sup>b</sup>	1.7 $\pm$ 0.6 (140) <sup>b</sup>	<0.001

8

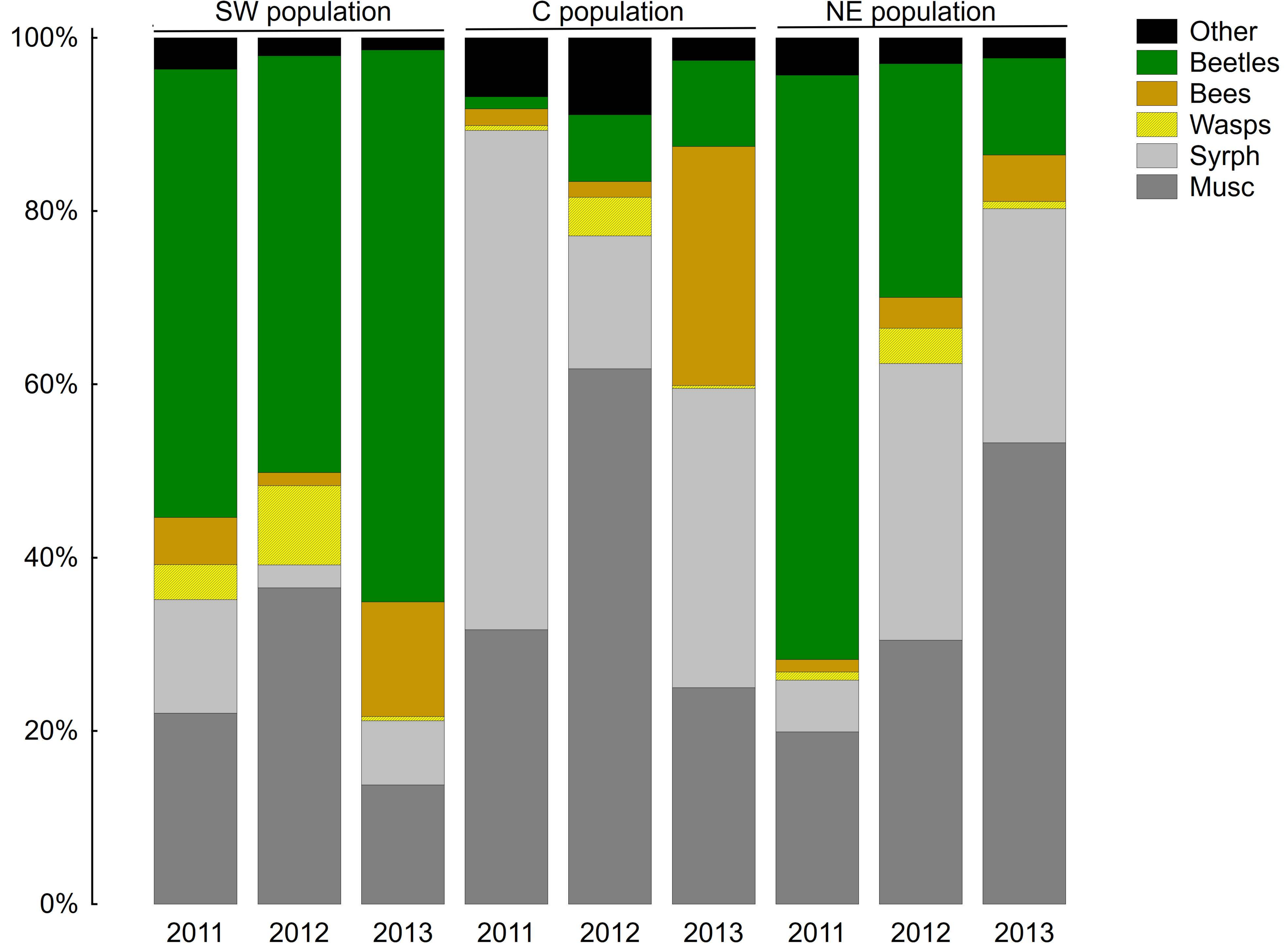
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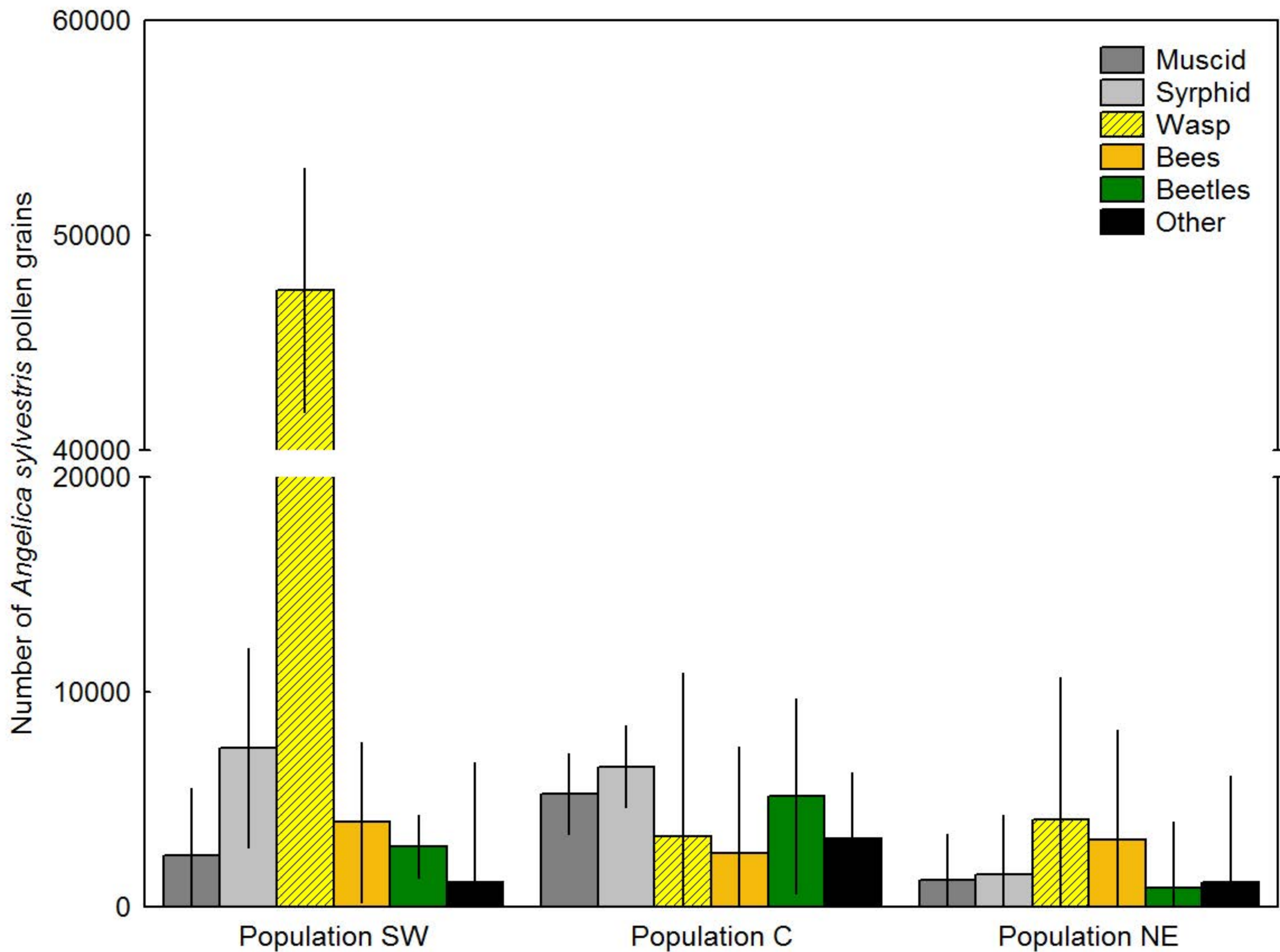
**Table 2.** Average seed-set ( $\pm$ SD) for three source populations over the course of two years. Results for plants of various origin were compared within each population using Kruskal-Wallis ANOVA. Numbers in brackets indicate sample size.

Plant origin	SW	C	NE	p
Population SW				
2015	0.86 $\pm$ 0.06 (10)	0.88 $\pm$ 0.07 (10)	0.79 $\pm$ 0.19 (10)	ns
2016	0.91 $\pm$ 0.10 (5)	0.92 $\pm$ 0.11 (7)	0.80 $\pm$ 0.21(7)	ns
Population C				
2015	0.85 $\pm$ 0.05 (3)	0.86 $\pm$ 0.07 (10)	0.89 $\pm$ 0.05 (10)	ns
2016	0.97 $\pm$ 0.04 (7)	0.69 $\pm$ 0.38 (8)	0.82 $\pm$ 0.28 (7)	ns
Population NE				
2015		0.86 $\pm$ 0.03 (2)	0.74 $\pm$ 0.04 (3)	ns
2016	0.79 $\pm$ 0.31 (9)	0.80 $\pm$ 0.30 (8)	0.70 $\pm$ 0.32 (8)	ns

1    **Supplementary Information. Table. A.** Body pollen loads of insect visitors to *Angelica*  
2    *sylvestris* umbels in three study populations SW, C and NE. For year: 1=2011, 2=2012,  
3    3=2013; for population: 1=SW population, 2=C population, 3=NE population; for umbel  
4    sexual phase: 1=male, 2=female; for visitor morphogroup: 1=muscid flies, 2=syrphids,  
5    3=wasps; 4=bees, 5=beetles, 6=Other; N denotes number of samples.



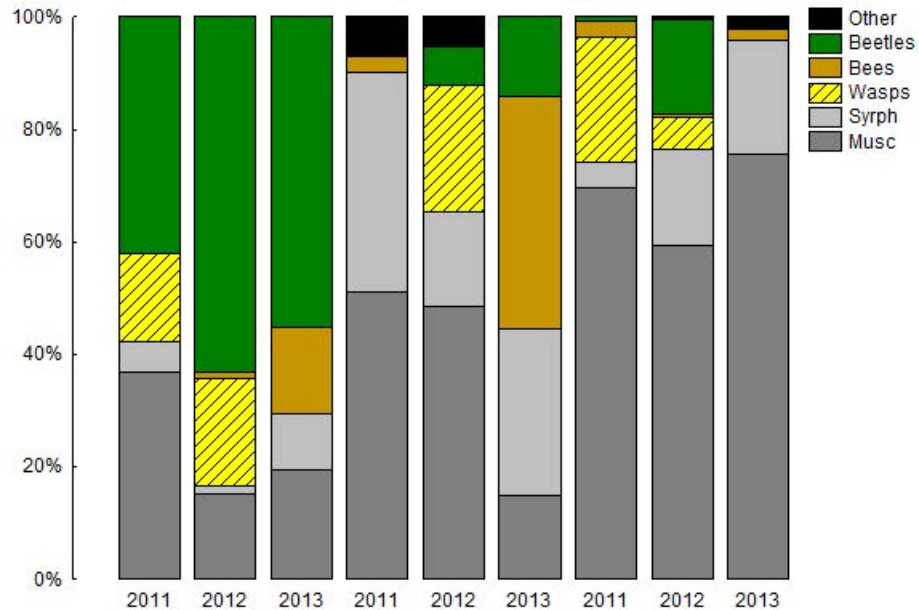


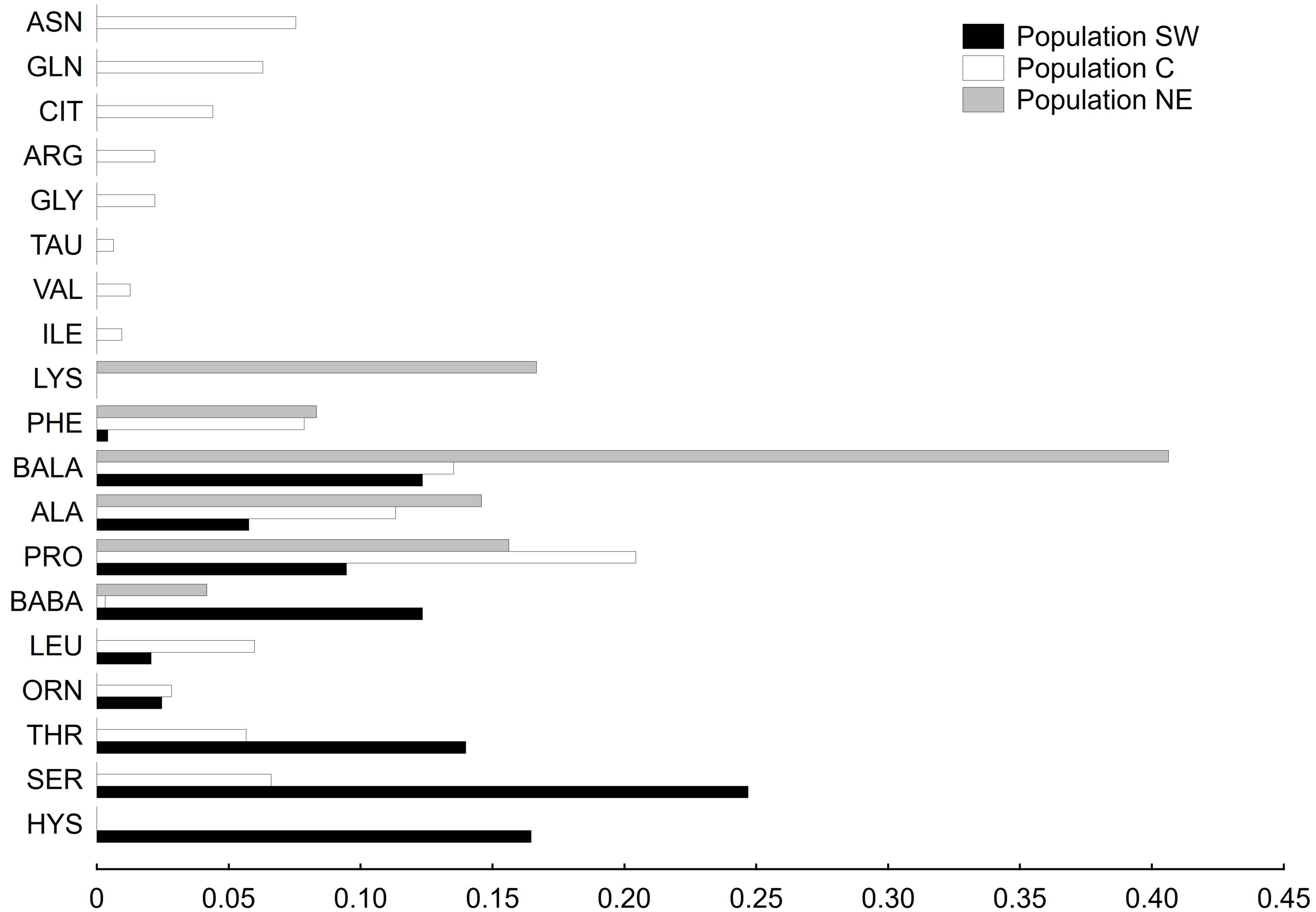


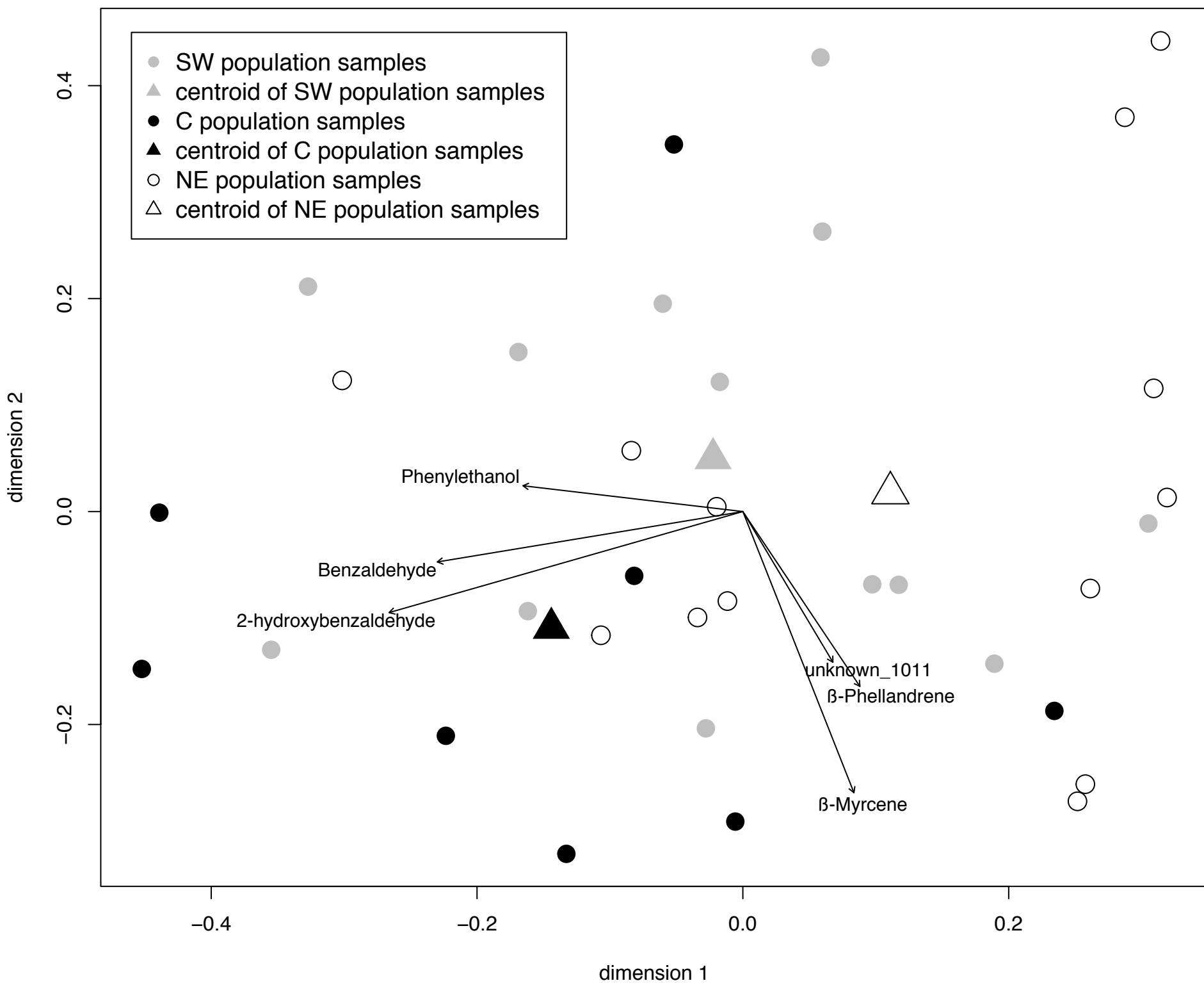
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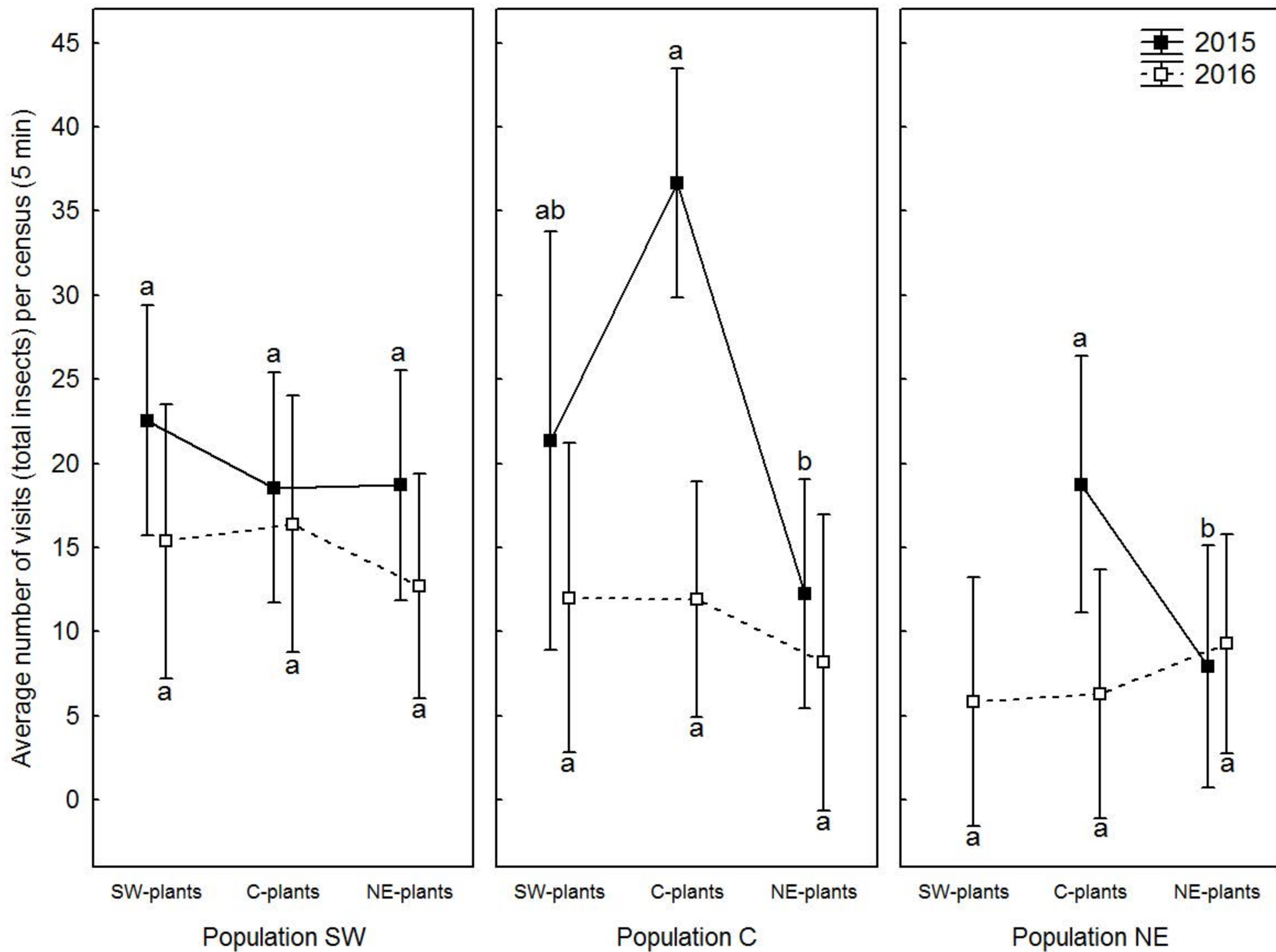
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NE





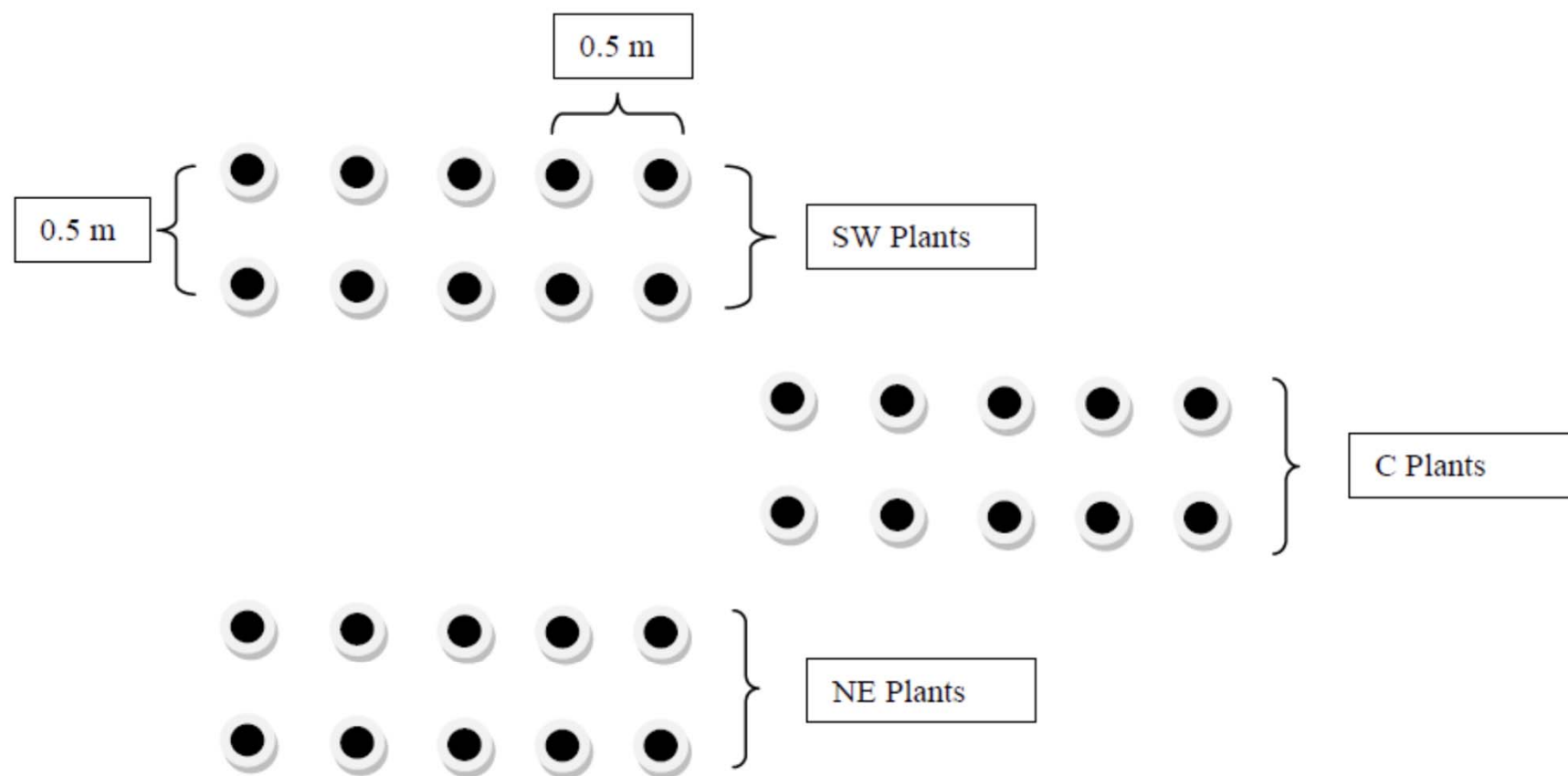




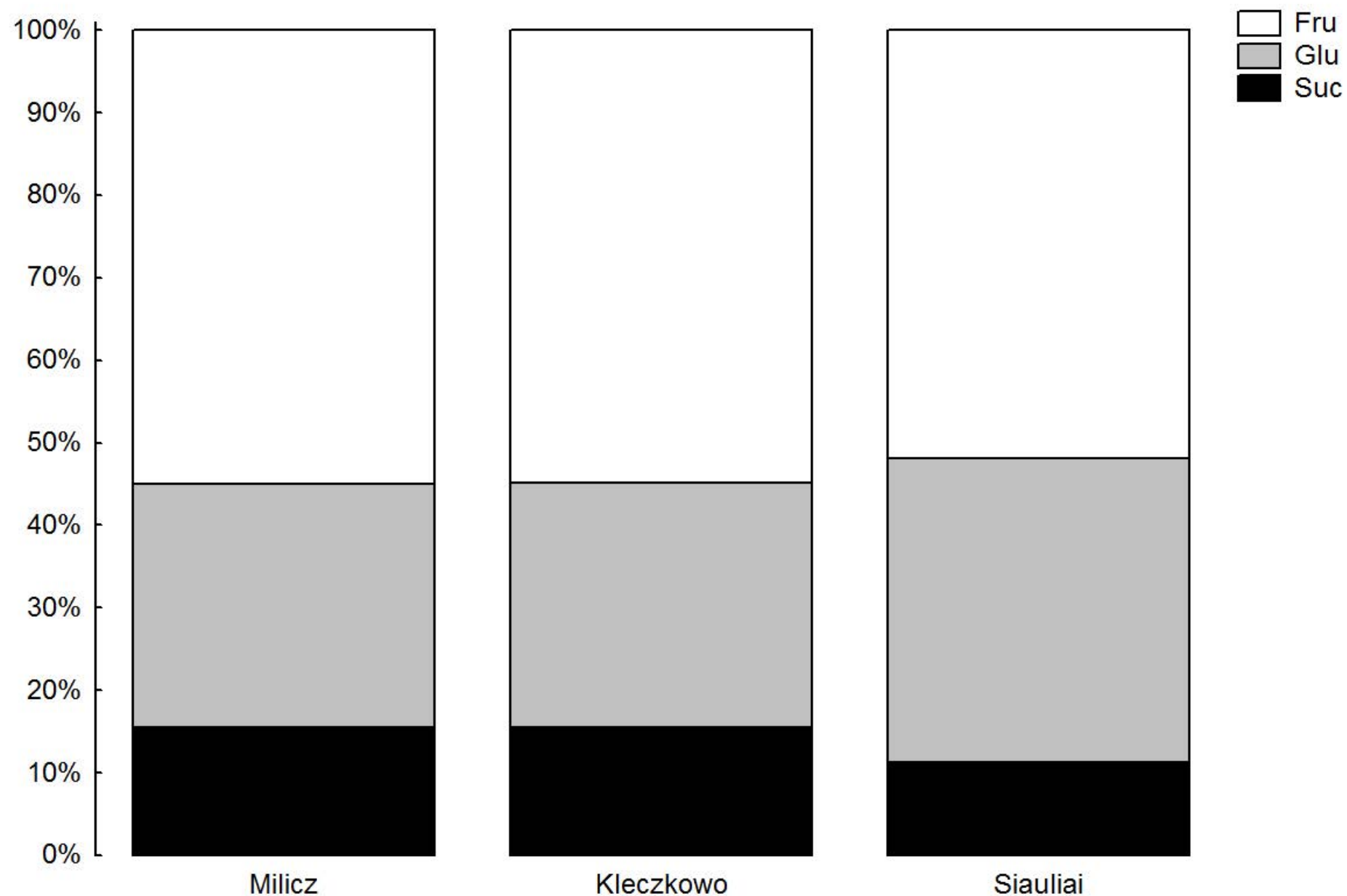
## Supplementary figures

Fig. B. supplementary material. Inflorescence penetration ratio (= proportion of visited umbellets in an umbel) by various visitor morphogroups for three study populations over three years of study. Results of Kruskal-Wallis ANOVA are shown. Error bars indicate standard deviation of the means. Vesp = wasps, Bee = bees, Syrph = hoverflies, Musc = Muscoid flies, Col = beetles.

Fig. C. Percentage proportion of sucrose, glucose and fructose in nectar from *Angelica sylvestris* plants derived from three investigated populations (SW, C and NE).



**Fig. A; supplementary materials.** Arrangement of potted plants during transplantation experiment. The same scheme was used in each of the source populations.



*Angelica sylvestris* nectar sugar composition, expressed as a relative % of Fructose (Fru), Glucose (Glu) and Sucrose (Suc) on total sugars.