ANNALS OF BOTANY
Founded 1887

PART OF A SPECIAL ISSUE ON ECOLOGY AND EVOLUTION OF PLANT REPRODUCTION

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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• **Background and Aims** In terms of pollination systems, umbellifers (plants of the carrot family, Apiaceae) are regarded as 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. Of particular interest was whether populations of an umbellifer species varied in pollinator assemblages and whether this could lead to local specialization of the pollination system. We also explored whether variation in pollinator assemblages was associated with variation in floral traits, and whether this variation influences reproductive output.
• **Methods** The focus was on *Angelica sylvestris*, a common European species visited by a taxonomically diverse insect assemblage. In three populations, located along an ~700-km transect, over three growth seasons insect visitors were identified, their effectiveness was assessed by surveying pollen loads present on the insect body, insect activity on umbels, nectar and scent composition was studied, and transplantation experiments were performed.
• **Key Results** The populations investigated in this study differed 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, analyses of body pollen loads and behaviour on umbels demonstrated functional equivalency of the visitor morphogroups, which is probably related to the fact that *A. sylvestris* bears few ovules per flower. The transplantation experiments confirmed that reproductive success was not related to the source of experimental plants and that the insects do not exhibit preferences towards local genotypes.
• **Conclusions** *Angelica sylvestris* is morphologically well adapted to ecological generalization, and there is little evidence that the surveyed populations represent distinct pollination ecotypes. Most likely, the observed variation in floral characters can be interpreted as 'adaptive wandering'. Specialization in this family seems possible only under very special circumstances, for example when the pollinator community comprises insect visitor groups that clearly differ in their pollination capacity (e.g. due to differences in their functional morphology) and/or have different perceptional biases (e.g. for colour or scent). However, the barrier to the evolution of morphological adaptations resulting in the fine-tuning of the flower towards particular pollinator types may arise from the architectural constraints on the floral bauplan that make umbellifers so uniform in their floral displays and so successful in attracting large numbers of pollinators.

Key words: Adaptive wandering, cantharophily, floral scent, generalization, myophily, nectar 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.*, 2014*a*; 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). For

specialization to occur, it is necessary that the observed groups of insect visitors differ in their capacity to pollinate. This can be achieved by variations in the abundance (quantity component of pollination) and effectiveness on a flower (quality component of pollination), together with preferences towards, or mechanical compatibility with, some floral phenotypes. As a result, different pollinator groups are able to variously affect plant fitness (Gómez and Zamora, 2006). 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). Another

important argument against obligate specialization is based on the evidence that plant–pollinator interactions are highly variable across time and space, both qualitatively and quantitatively (Schemske and Horvitz, 1984; Herrera, 1988, 1989; Ollerton, 1996; Waser *et al.*, 1996; Gómez and Zamora, 1999, 2006; Price *et al.*, 2005; Cosacov *et al.*, 2008; Artz *et al.*, 2010; Castro *et al.*, 2013). Therefore, some authors have suggested that generalist pollination actually 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; Johnson and Steiner, 2000; Fenster *et al.*, 2004; Willmer, 2011; Padyšáková *et al.*, 2013; Bartoš *et al.*, 2015). There are, however, caveats to this reasoning, since most data published on the evolution of pollination systems are based on studies of relatively specialized plants (at least phenotypically), whereas information concerning phenotypic generalists, i.e. plants with flowers easily accessible for a wide taxonomic array of visitors, is relatively scarce (Ollerton *et al.*, 2007), which only provides a partial picture of the role of pollinators in the evolution of flowers. Furthermore, we generally lack information on spatial diversity of pollination systems, and 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, 2010). In fact, there is increasing evidence for the evolution of pollination ecotypes adapted to local pollinator assemblages (Johnson, 2006; Perez-Barrales *et al.*, 2007; Anderson *et al.*, 2009; Armbruster and Muchhala, 2009; Gómez *et al.*, 2009*a*, 2015; Johnson, 2010; Cosacov *et al.*, 2014; Van der Niet *et al.*, 2014*b*; Yamada *et al.*, 2014). Therefore, as postulated by Thompson (2005), many plants that appear to be specialists at the population level may in fact be generalists at the species level. However, in contrast to specialist plant species, where differences in pollinator fauna are of a qualitative character, generalized pollination systems differ mostly in the relative abundances of various pollinators, and evolution of their pollination niches and drivers of floral divergence are largely unknown (Gómez *et al.*, 2015, and references cited therein).

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

One of the plant families often associated with generalist pollination systems is Apiaceae (Faegri and van der Pijl, 1966; Proctor *et al.*, 1996; Corbet, 2006; Zych *et al.*, 2007), a taxon characterized by a high degree of floral and inflorescence uniformity (Bell, 1971; Bell and Lindsey, 1978). Indeed, Olesen *et al.* (2007) estimated that several members of Apiaceae are amongst the top ten plant generalists, based on the number of insect taxa that visited their flowers. These plants included *Angelica sylvestris*, which was visited by at least 245 insect species occurring within the plant's geographical range (Ellis and Ellis-Adam, 1993). This general statement, however, may not necessarily be true for all members of the Apiaceae, since more

specialized plant–pollinator relationships have been described for other umbelliferous species (Lindsey, 1984; Zych, 2007; Niemirski and Zych, 2011; Cursach and Rita, 2012*b*; Zych *et al.*, 2014). These relationships may result from variation in many subtle, pollination-related characters that, in certain cases lead to 'cryptic specialization' of pollination systems (Bell, 1971). Such characters include, for example, umbel density (Bell and Lindsey, 1978; Lindsey and Bell, 1985; Bisht *et al.*, 2008) and sex ratio (Pickering and Hill, 2002), the degree of dichogamy (Cruden and Hermann-Parker, 1977; Webb, 1981; Schlessman and Barrie, 2004), nectar composition and secretion (Lindsey and Bell, 1985; Stpiczyńska *et al.*, 2015) and floral scent profiles (Borg-Karlson *et al.*, 1994; Tollsten *et al.*, 1994; Tollsten and Øvstedal, 1994). However, to address the issue of (local) specialization of pollination systems in Apiaceae, a better sampling of spatial and/or temporal variation in the pollination of natural populations of this large and economically important family is necessary. Unfortunately, we are aware of only ten studies that address these questions (Lindsey and Bell, 1985; Kaye and Kirkland, 1994; Lamborn and Ollerton, 2000; Pérez-Bañón *et al.*, 2007; Zych, 2007; Davila and Wardle, 2008; Danderson and Molano-Flores, 2010; Niemirski and Zych, 2011; Cursach and Rita, 2012*a*; Zych *et al.*, 2014), only five of which concern both phenomena. The general picture that arises from the aforementioned studies is intriguing. For example, *Daucus carota* (Lamborn and Ollerton, 2000) and Australian *Trachymene incisa* (Davila and Wardle, 2008) exhibited great fluctuations in pollinator assemblages. This contrasts markedly with data obtained for the reputedly supergeneralist *A. sylvestris*, which is visited by numerous insect taxa, but pollinated only by a taxonomically relatively narrow group of muscoid and syrphid flies (Niemirski and Zych, 2011). Furthermore, the composition of the pollinator assemblage for *A. sylvestris*, like that of another umbellifer, namely *Heracleum sphondylium* (Zych, 2007), remained constant in subsequent years. Therefore, it would appear that, despite their superficial uniformity, umbellifers display a whole range of different pollination strategies, perhaps even including 'cryptic specialization' (Bell, 1971), with certain subtle floral features attracting (or repelling) particular visitors to the flower. More data, however, relating to both temporal and spatial variation in the pollination system, are required if we are to investigate this hypothesis. To this end, we undertook assessment of the spatiotemporal variation present in the generalist pollination system. For the above reasons, we selected *A. sylvestris* as a model plant for this study. This species has a broad distribution range (Cannon, 1968) and is among the very few European Apiaceae taxa that have been 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 the questions of whether *A. sylvestris* populations varied in pollinator assemblages and whether this could lead to local specialization of the pollination system. We also explored whether variation in pollinator assemblages was associated with variation in floral traits, and whether this variation influences reproductive output.

MATERIALS AND METHODS

Angelica sylvestris

Angelica sylvestris (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 ~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 (~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. The nectar of *A. sylvestris* is hexose-rich and composed of sucrose, glucose and fructose, as well as a small amount of amino acids. Nectar sugar concentration is similar for both floral sexual phases, but nectar production is male-biased, being >3-fold greater than in female-phase flowers (Stpiczyńska *et al.*, 2015). This, however, does not appear to result in discrimination against the 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 north-east Poland they are chiefly pollinated by a narrow assemblage of muscid and syrphid flies (Niemirski and Zych, 2011).

Study populations

In each of three years (2011–13) we conducted field observations and insect sampling for three Central European *A. sylvestris* populations located along an ~700-km south-west/north-east transect: (1) Milicz, Lower Silesia region, south-west Poland, 51°30′36″ N, 17°18′23″ E, 132 m a.s.l., hereafter referred to as the SW population; (2) Kleczkowo, Mazovia region, north-east Poland, 53°02′33″ N, 21°51′37″ E, 106 m a.s.l., hereafter referred to as the central (C) population [the same population was studied earlier by Zych and co-workers (Zych *et al.*, 2007; Niemirski and Zych, 2011)]; and (3) Šiauliai, Šiauliai region, north Lithuania, 55°47′52″ N, 23°18′27″ E, 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 July and August in 2011–13, which is the peak flowering time for *A. sylvestris* in these regions. For insect observations and sampling, a method slightly modified from 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 (six for female-phase umbels and six for male-phase umbels), and during 2012–13 we completed 24 rounds annually (12 for female-phase umbels and 12 for male-phase umbels). Each round consisted of three phases: random selection of umbels; video recording (15 min, using an HDRXR106 digital camera; Sony, Japan); and insect sampling (15 min, using an entomological net or directly into plastic vials), totalling 90 h of observations and insect sampling over 3 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.

FIG. 1. (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.

Since earlier work conducted in the C population revealed that before 0800 h and after 1900 h flowers of *A. sylvestris* were visited by insects very rarely [in particular, no typically nocturnal insects (e.g. sphingid moths) were recorded (Niemirski and Zych, 2011)], we decided to restrict our observations to the parts of the day with the most intensive insect activity. Therefore, 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 single populations, observations lasted for at least 3 full days. More often, however, inclement weather (strong winds or rain), led to a longer observation period, and 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. Our observations were restricted to primary umbels because in most umbellifers, including the genus *Angelica*, they 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 (Carvalheiro *et al.*, 2008; Cursach and Rita, 2012*a*), 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 taxonomic 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 and 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 (~3–4 mm^3) 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 subsampled (all pollen grains were scored for nine areas evenly

distributed over the coverslip) 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

To estimate the pollination effectiveness of insect visitors, we adopted the approach used, for instance, by Zych (2002, 2007), Niemirski and Zych (2011) and Bouman *et al.* (2017). This included 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 is the importance of insect species *x*, *V* is abundance (number of recorded visits of species *x* + number of captured individuals of species *x*)/(total number of recorded visits + total number of captured individuals), *U* is umbel penetration ratio (mean number of umbellets visited by species *x* within an umbel/mean number of umbellets in an average umbel in the population surveyed) and *PL* is average pollen load (number of pollen grains) carried by an individual of species *x*.

We calculated *I* 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 20–30 whole individual plants and mounted them on herbarium sheets. Later in the laboratory, using stereoscopic binoculars, for each plant we counted the number of umbellets in primary (main) umbels (equivalent to the size of an inflorescence) and the number of male/bisexual flowers. The number of bisexual flowers was calculated as the sum of developed fruits and non-pollinated flowers (ones that failed to form fruit). In order to assess seed set, we used the pooled counts from three randomly chosen umbellets from each main umbel. Seed set was calculated as the number of seeds divided by the number of bisexual flowers. We focused on inflorescence features since, in the Apiaceae, umbels rather than the minute flowers are recognized as units of attraction (Bell and Lindsey, 1978). Additionally, in 2012 we collected five fully ripe seeds from the main (first-order) umbel of each plant and weighed them using an analytical balance (AS 60/220/C/2 RADWAG; Radom, Poland).

Nectar sampling

In order to check for differences in floral rewards between populations, samples of nectar were collected in 2012 and 2013 from 15–25 flowers at the male stage and, owing to the smaller

volume of available nectar, from 30–40 flowers at the female stage. The small volume of nectar produced meant that we were only able to collect 23 samples in 2012 (9, 6 and 8, respectively from SW, C and NE plants) and 39 in 2013 (13 samples for each population). The nectar was collected using microcapillary pipettes of known mass, and subsequently expelled from the pipette onto a refractometer prism RL-4 (PZO, Warszawa, Poland), and nectar sugar concentration was calculated and expressed as a percentage weight of nectar.

In order to determine the composition of nectar sugars during both floral sexual stages, nectar from 50 flowers for each stage of development was collected using micro-pipettes, pooled and analysed by isocratic HPLC in conjunction with an LC1 Waters system. A 20-µL aliquot of both sample and standard solution was injected. Water (MilliQ, pH 7), with a flow rate of 0.5 mL min⁻¹, was used as the mobile phase. Sugars were separated in a Waters Sugar-Pack I column (6.5–300 mm) maintained at 90 °C, and identified by a refractive index detector (Waters 2410). The fructose, glucose and sucrose contents were determined and expressed as percentages of total sugars.

Amino acid analysis of a 10-µL sample of nectar collected and pooled from 50 flowers was performed by gradient HPLC using an ion-exchange Novapak C18 (15 mm × 4.6 mm) cartridge, with guard column maintained at 37 °C, and a Waters 470 scanning fluorescence detector (excitation at 295 nm, detection at 350 nm). A solvent composed of triethylammonium (TEA) phosphate buffer (pH 5.0) mixed with a 6:4 acetonitrile–water solution was used as the mobile phase at a flow rate of 1.0 mL min⁻¹. According to the AccQtag protocol (Waters), the selected volume of each reconstituted sample was amino acid-derived (Cohen and Micheaud, 1993) with 6-aminoquinolyl-N-hydroxysccinimidyl carbamate (AQC) fluorescent reagent and 0.02 ʍ borate buffer (pH 8.6). In addition to all the protein amino acids, standards of β-alanine, citrulline, ʟ-homoserine, α-aminobutyric acid (AABA), γ-aminobutyric acid (GABA), hydroxyproline, ornithine and taurine were also used.

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

Scent sampling

The same common-garden approach was used for scent sampling in 2016. Dynamic headspace scent samples were collected from the main inflorescence in full bloom from 20–25 plants per population following the method described by Kuppler *et al.* (2016). Samples of floral scent were collected in the laboratory from potted plants from each population. The umbels were enclosed within a polyester oven bag (Toppits®, Germany) for

15 min and the emitted volatiles were then trapped on 1.5 mg of Tenax (mesh 60–80; Supelco, Bellefonte, PA, USA) and 1.5 mg of Carbotrap B (mesh 20–40; Supelco) in a quartz vial (Varian; length 15 mm, inner diameter 2 mm) for 2 min using a membrane pump (G12/01 EB; ASF Rietschle-Thomas, Puchheim, Germany) with a flow rate of 200 mL min⁻¹. All samples were collected between 1000 and 1400 h. Scent samples were analysed using an automatic thermal desorption system (TD-20; Shimadzu, Japan) coupled with GC–MS (model QP2010 Ultra EI; Shimadzu, Japan). The GC–MS system was equipped with a ZB-5 fused silica column (5 % phenyl polysiloxane; 60 m long, inner diameter 0.25 mm, film thickness 0.25 µm; Phenomenex) and the column flow (carrier gas: helium) was set to 1.5 mL min⁻¹. The GC oven temperature started at 40 °C (split ratio 1:1), then increased by 6 °C per minute to 250 °C and was then held constant for 1 min. The MS interface worked at 250 °C. Mass spectra were taken at 70 eV (in EI mode) from *m/z* 30 to 350. The GC–MS data were processed using the GCMSolution package (Version 2.72; Shimadzu). Compounds were identified by comparison of the mass spectra and Kovats retention index with standard compounds, which are commercially available. Alternatively, compounds were identified using the mass spectral libraries Wiley 9, Nist 2011, FFNSC 2, Essential oils and Adams 2007, as well as the database available in MassFinder 3. The compounds found in the flowers were compared with those present in the blanks (empty oven bags) so as to determine which compounds were specifically emitted by inflorescences.

Transplantation experiment

In order to check the performance of plants from various sources in native versus 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. Plants for potting were collected each year from source populations in spring, potted in compost 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 (eight plants from each of SW, C and NE). Pots with plants were arranged according to the scheme shown in Supplementary Data Fig. A. During flowering, insect activity on the primary umbel of each plant was recorded using digital video cameras. Since adaptation to local conditions may arise both through the male and female functions, this was performed twice for each plant (in male and female phase), and video recordings lasted 5 min each. Video recordings were analysed in the laboratory 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 numbers of fruits, non-pollinated female flowers and male flowers in primary umbels were scored.

Statistics

Statistica 13.1 (Dell) was used for most statistical calculations. To compare 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 resulting 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 a normal distribution. Data on fruit set and nectar concentration could not be successfully transformed, and therefore for comparisons a generalized linear model implementing binary logistic distribution was used in the first case, and non-parametric Kruskal–Wallis ANOVA in the other. Temporal constancy of insect assemblages within a particular population was tested using the G-test, with the proportion of visits in the first year set as an expected proportion for subsequent years.

To test whether nectar amino acid and floral scent composition differed between populations, we used 'random forest' analysis (Breiman, 2001) implemented in the R package randomForest (R Development Core Team, 2011). This machine-learning algorithm made it possible 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 ~ sqrt(variables) randomly selected at each node. Random forest analysis returns a confusion matrix that shows the number of correctly assigned samples for each population (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 AA or scent component strongly separates the populations. Results of scent analysis were subject to non-metric multidimensional scaling (NMDS) based on Bray–Curtis distances of quantitative scent emissions using the R package vegan (Dixon, 2003).

RESULTS

Insect visits and behaviour

During the course of 3 years, we recorded 8477 insect visits to our study plants. The overall visit frequency was 32 ± 25 visits per census (15 min), and this did not differ significantly between populations (data pooled over 3 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. Generally, 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 even less consistent. In 2011, 67 % of visits were 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 the SW, C and NE populations), 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 the SW, C and NE populations; in years 2011–12 for all populations ≤5 %). The observed patterns, however, were highly variable between populations and years (Fig. 2; for all comparisons P < 0.05, G-test).

Insect behaviour on umbels (calculated as the proportion of visited umbellets in an umbel) was variable across both years and populations, and showed no particular pattern (Supplementary Data Fig. B) (since the data on insect behaviour on umbels were to be used to compare visitation patterns of particular insect guilds, we employed non-parametric 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 the SW, C and NE populations) and found large variation in pollen load between individual members of a particular morphogroup (averages for all recognized morphogroups for three populations over the three study years are presented in Supplementary Data Table S1). 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 the SW population.
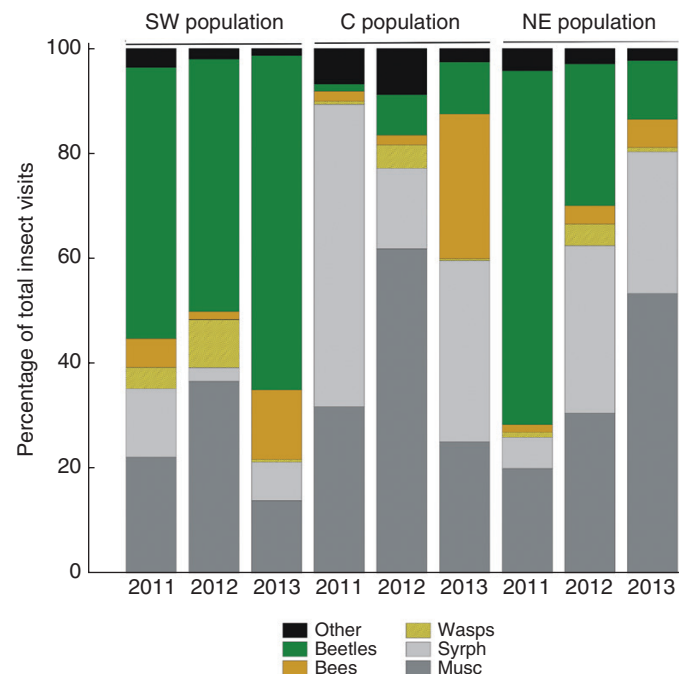

Fig. 2. Insect visits to umbels of *Angelica sylvestris* for years 2011–13 in the three study populations (SW, C and NE) expressed as the percentage of total visits for a given year within a particular population. Data are based on captures and video records. Musc, Muscoid flies; Syrph, Syrphidae.

By comparison, the largest 'other' pollen load, of 81 037 grains, was found on a beetle netted in 2011 on a female-phase umbel in the C population. Most of the analysed loads (~66 %) were composed of both types of pollen, and the quantities of *A. sylvestris* and 'other' pollen were positively correlated (Pearson's product moment correlation coefficient $r^2 = 0.1896$, $P < 0.005$). Fifteen percent of all captured insects carried no pollen at all, whereas 15 and 4 % of loads were composed of only *A. sylvestris* and 'other' pollen grains, respectively. Overall, both the largest *A. sylvestris* load and the largest 'other' pollen load were carried by seldom-observed wasps (mean ± s.d. 22 801 ± 76 655 and 3 804 ± 9 610 pollen grains, respectively). 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 unequal *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 3 years of sampling, the most important pollinators were flies (muscids and syrphids), beetles and hymenopterans (bees and wasps), with very marginal contribution from other insect groups. As in the visitation data, the relative contributions of particular insect groups remained highly variable between populations and across years (Fig. 4). Generally, flies were the key pollinators of plants in the C and NE populations (IC range 45–96 %), whereas beetles contributed most to the pollination of SW plants, but played a rather marginal role in the pollination of the two remaining populations (IC from 0 to a maximum of 17

% in 2012 for NE). The results for hymenopterans (bees and wasps) were even more variable across years, and the morphogroups usually replaced each other, i.e. if the contribution of bees was noteworthy (as in 2013 for SW or C), wasps were effectively absent and vice versa (2011 in SW or NE, and 2012 in C).

Floral characters and reproductive success of populations

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

Nectar

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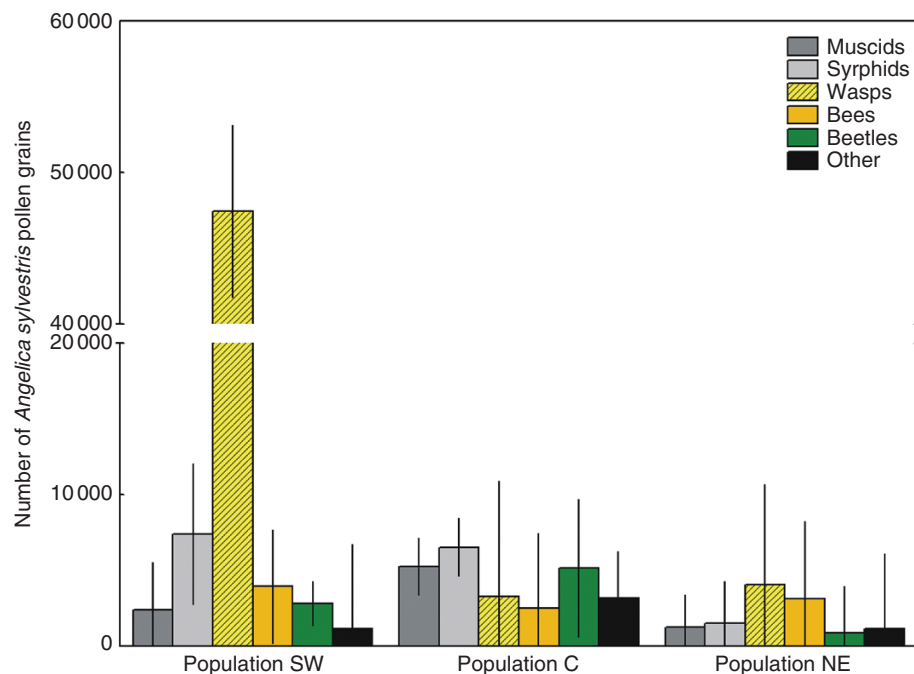

FIG. 3. Average body pollen loads carried by members of a particular pollinator morphogroup. Error bars indicate 95 % confidence limits of the mean. Data were pooled for study years and umbel sexual phases. Note the gap in the *y*-axis.

In 2012, plants from different populations produced nectar of similar sugar concentration [overall, the 2012 mean was 26.2 ± 14.4 %; pooled data from male- and female-phase umbels; Kruskal–Wallis ANOVA$_{2012}$, $H(2, N = 23) = 3.450345$, $P = 0.178$]. However, nectar sugar concentration varied between populations in 2013 [Kruskal–Wallis ANOVA$_{2013}$, $H(2, N = 39) = 9.150101$, $P = 0.010$]. The lowest sugar concentration for 2013 (mean ± s.d. 15.4 ± 5.5 %) was found in C plants, but was significantly higher (23.1 ± 6.4 %) for SW plants (sugar nectar concentration for NE plants, 20.9 ± 6.5 %, did not differ significantly from that of either group).

The SW and C plants produced nectar that was relatively richer in amino acids (0.12 ± 0.07 and 0.16 ± 0.08 mm, respectively), whereas the amino acid concentration for the NE plants was ~3-fold lower (0.05 ± 0.01 mm). Unfortunately, due to low nectar volumes, we were able only to analyse 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 AAs in the collected samples of nectar (Fig. 5). Only alanine, proline, phenylalanine, β-alanine and β-aminobutyric acid (BABA) were detected in at least one sample from each of the three populations, and many AAs were population-specific. For example, asparagine, glutamine, citrulline, arginine, glycine, taurine, valine and isoleucine were recorded only for the C population, whereas lysine was unique to NE and histidine to SW samples. Random forest analysis assigned all SW samples to the NE population, indicating that these populations are not well separated and that C plants differed in nectar AA profiles from individuals constituting the other two populations. The estimated error rate for the whole data set was 66.67 %, indicating that two-thirds of the samples were not correctly assigned to the populations.

Floral scent emissions

In total, we detected 44 floral scent compounds in 34 samples collected from plants derived from the three study populations. Our populations overlapped for the presence/absence of most compounds (Supplementary Data Table S2), 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 the C and SW populations received slightly higher random forest class errors: 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: R2 = 0.15, $P = 0.033$).
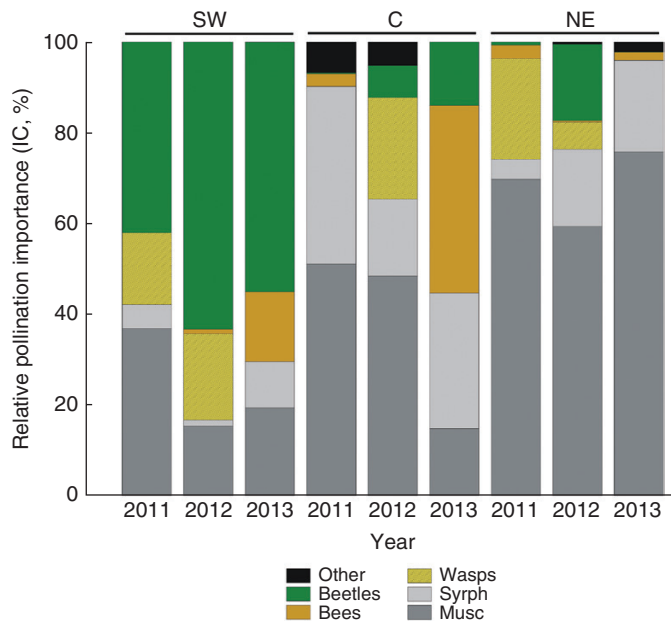

FIG. 4. Relative pollination importance of insect visitors to umbels of *Angelica sylvestris* in years 2011–13 for the three study populations: SW (Milicz, southwest Poland); C (Kleczkowo, north-east Poland); and NE (Šiauliai, north-east Lithuania), based on visitation data, pollen loads and behaviour on flowers. Musc, muscoid flies; Syrph, Syrphidae.

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

TABLE 1. *Reproductive characters of plants from study populations. Number of umbellets was treated as a proxy for inflorescence size. Data are given for primary (main) umbels and presented as mean ± s.d. (sample size). Means with different letters are different at* P < 0.05 *(post hoc* Tukey HSD test for unequal N*). Data on fruit set could not be successfully transformed, and therefore a generalized linear model implementing binary logistic distribution 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*

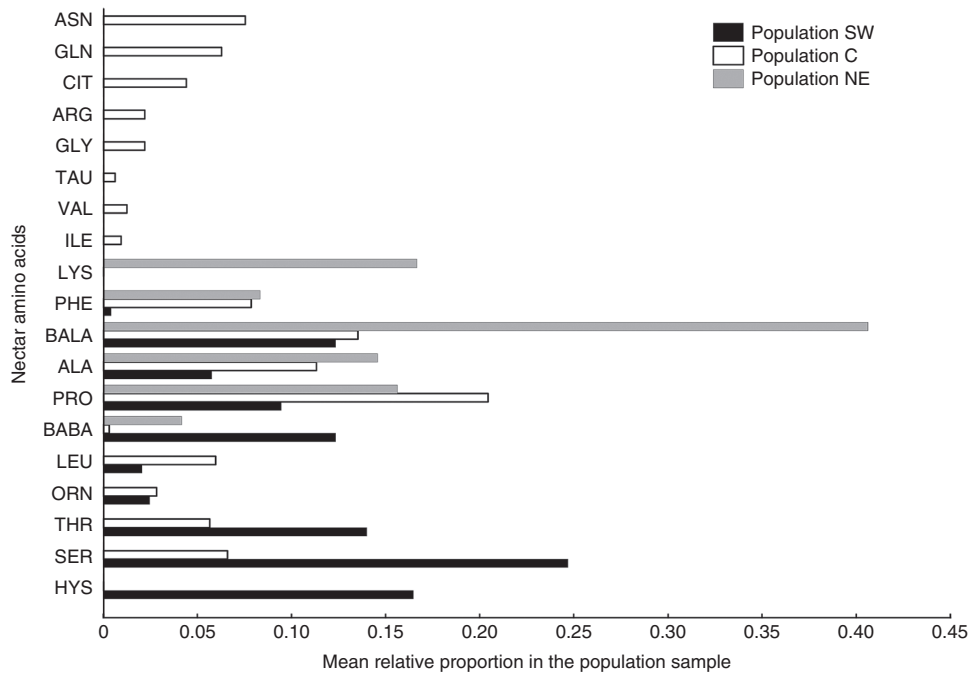|	SW (Milicz)	C (Kleczkowo)	NE (Šiauliai)	*P*
Number of umbellets, 2012	30 ± 7 (28)[a]	25 ± 7 (27)[b]	36 ± 8 (12)[a]	<0.001
Number of umbellets, 2013	22 ± 5 (29)	30 ± 7 (14)		<0.001
Mean fruit set, 2012	0.99 ± 0.02 (24)	0.95 ± 0.14 (27)	0.95 ± 0.14 (12)	ns
Mean fruit set, 2013	0.95 ± 0.10 (29)	0.98 ± 0.06 (14)		ns
Mean seed mass (mg)	2.1 ± 0.6 (175)[a]	1.8 ± 0.6 (125)[b]	1.7 ± 0.6 (140)[b]	<0.001

ns, not significant.

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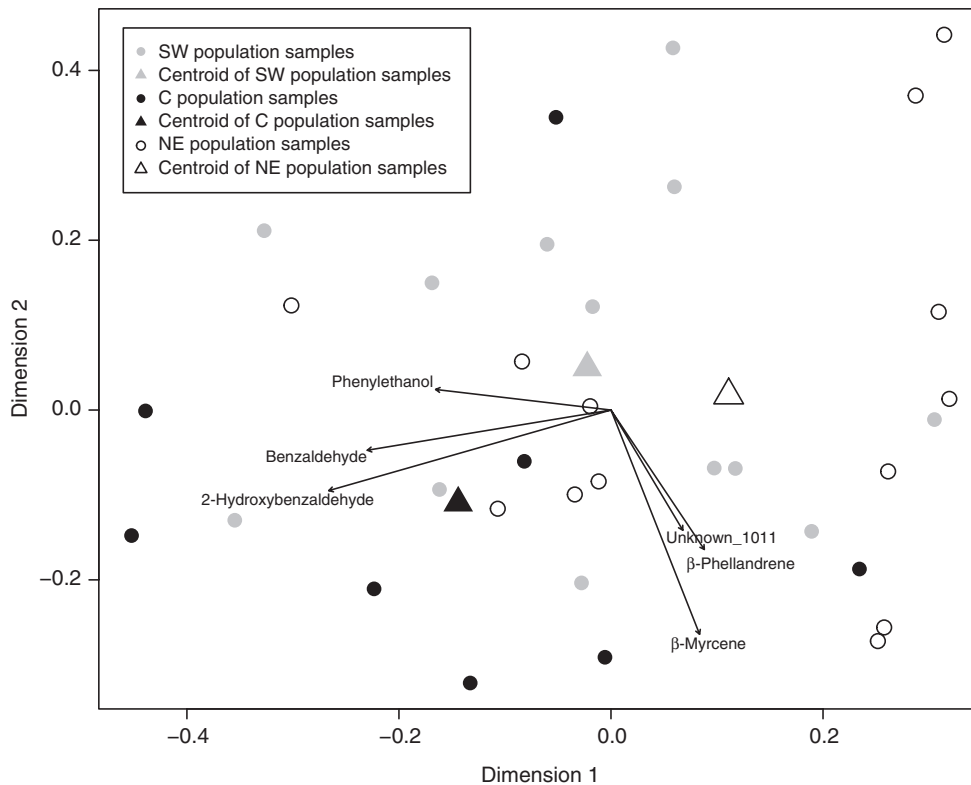

Fɪɢ. 6. Differences in scent composition of *Angelica sylvestris* flowers from three populations. Ordination shows results of NMDS based on Bray–Curtis distances on quantitative emission rates of flowers. Each sample is displayed as a circle and the population centroids are shown as triangles. Volatile organic compounds that significantly (*P* < 0.01) correlated with samples in the 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.

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. The only exception was recorded in 2015, when in the C and NE populations C plants were visited more frequently than NE plants [Kruskal–Wallis ANOVA, C population, $H(2, N = 46) = 6.8110$, $P = 0.03$; 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]. Furthermore, no increase in visitation to male-phase umbels of local plants was observed (Fig. 7).

Seed set for our experimental (potted) plants was lower than in 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 geographical and temporal variation in the pollination system of *A. sylvestris*, which provides little evidence that the surveyed populations represent distinct pollination ecotypes. Although visitor assemblages remained similar qualitatively, i.e. all main morphogroups

were present in all three study populations, the contribution of a given pollinator morphogroup was variable between populations and over the years. In general, however, the SW population was more often visited and pollinated by beetles, whereas the remaining two (C and NE) were visited rather by dipterans. Interestingly, the results for the 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 ~90 % of pollination events. This suggests that the temporal consistency of pollinators in the case of our study species may be maintained over longer periods, a necessary precondition for the local specialization of populations depending on pollination by flies and beetles. However, 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*, in which most visitor groups, including dominant beetles and flies, seem to be equally effective on flowers, at least where insect body pollen loads are concerned. Similar spatial (and temporal) turnover of equivalent pollinators can be observed for some other umbellifers, such as *D. carota*, because various populations of this species in the UK are mainly visited
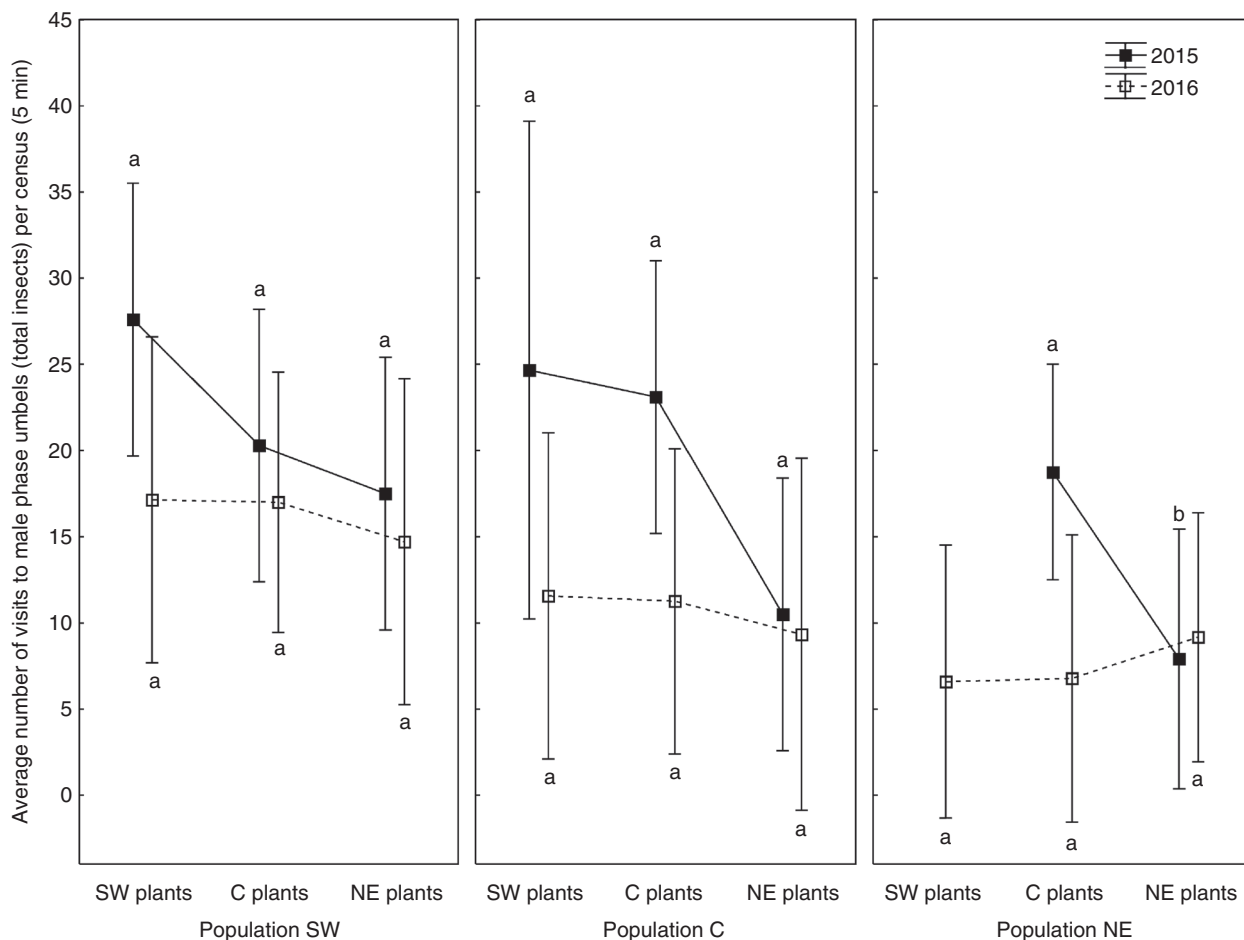

FIG. 7. Average frequency (and 95% confidence interval of the mean) of total insect visits to male-phase umbels of experimental *Angelica sylvestris* plants in various population settings during the transplantation experiment over the course of 2 years. Means with different letters are different at $P < 0.05$ (Kruskal–Wallis ANOVA calculated for each site and study year).

TABLE 2. *Seed set, shown as average ± s.d. (sample size), for three source populations over the course of 2 years. Results for plants of various origins were compared within each population using a generalized linear model implementing binary logistic distribution*

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

ns, not significant.

by beetles (*Rhagonycha fulva*, which was also abundant in our study), sawflies (*Tenthredo* sp.) or dipterans carrying similar amounts of pollen (Lamborn and Ollerton, 2000). According to these authors, the plants rely on functionally similar groups that fluctuate over the years in terms of their abundance, but collectively do not differ in their importance.

The only marked exception in our study were wasps, whose body pollen loads in the SW population exceeded those of other pollinators by one order of magnitude. This result is probably due to the fact that, of the recorded insect visitors, Vespidae had the largest bodies. These insects, however, were also quite erratic visitors, both in SW and the other two populations, and were almost completely absent from all three study sites in 2013. This finding resembles that for a 3-year study of the umbelliferous plant *Ostericum palustre*, in which wasps were key pollinators for a single season and virtually absent for the rest (Zych *et al.*, 2014). Generally, wasps seem to be rather opportunistic visitors to Apiaceae flowers (Zych, 2002; Zych *et al.*, 2014), but their presence and sometimes aggressive behaviour can affect the performance of other pollinators. Fluctuations in the annual abundance of wasps may be caused by, for example, unfavourable climatic conditions (Archer, 2001). They are thus unlikely to exert a significant selective pressure on the studied system. Nevertheless, where other visitor groups are concerned, even the smaller average pollen loads were more than sufficient to pollinate *A. sylvestris* flowers, which contain only two ovules. Indeed, seed set was invariably close to 100 % in all studied localities, and the populations did not seem to be pollen-limited. In such situations, if plants bear few ovules per flower and thus require relatively few pollen 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), even further minimizes the probability that *A. sylvestris* could specialize 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 indicates 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,

which was probably caused by suboptimal growing conditions, we observed differences neither 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 the C and NE populations in 2015, which, however, did not result in decreased seed set.

Pollinators can also act as selective agents solely through an increase in male fitness, while female fitness remains unaffected (van der Niet *et al.*, 2014a). However, in *A. sylvestris* we found little evidence to support this hypothesis. In particular, no increase in insect visitation to male-phase umbels was observed either in the transplantation experiment or during observations in natural populations, and therefore this possibility seems of little significance.

In view of this, our finding that differences in insect visitor assemblages were generally not associated with changes in the floral characters of populations seems to confirm the very generalist pollination strategy of the investigated species. This similarity extends to floral rewards, especially nectar characteristics, which were generally constant across our study sites, at least as far as sugar 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 the overall nectar AA composition of the 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; Terrab *et al.*, 2007; Gijbels *et al.*, 2014). Nevertheless, like a previous study of *A. sylvestris* nectar (Stpiczyńska *et al.*, 2015), our survey showed that proline and alanine are core constituents of the nectar AA profile in all surveyed populations, but also confirmed that the nectar contains a substantial proportion of non-protein β-alanine, which in the SW population constituted >40 % of all nectar AAs. Alanine and proline are generally common nectar AAs (Baker, 1977), the latter being preferred by many pollinators, especially bees, probably because of its role in insect flight (Teulier *et al.*, 2016). Generally, insects seem to prefer high concentration of AAs, perhaps because of their alimentary value and specific taste (Baker, 1977; González-Teuber and Heil, 2009; Roy *et al.*, 2017), which might explain fewer visits to our experimental NE plants, which contained smaller quantities of AAs. This aspect of *A. sylvestris* pollination biology most certainly deserves further attention.

Regarding scent bouquets, our study plants produced floral odours that were mostly composed of various terpenoids and aromatic compounds, and resembled those found in earlier studies focused on *A. sylvestris* or other Apiaceae (Borg-Karlson *et al.*, 1994; Tollsten *et al.*, 1994). Such compounds are usually interpreted not as species-specific cues, but rather as a signal for a wide spectrum of insect visitors (Willmer, 2011). All volatile organic compounds found in *A. sylvestris* scent are common in floral bouquets. For example, 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, β-myrcene, β-phellandrene and phenylethanol. Some of these compounds can also be associated with nocturnal pollination (Dobson, 2006), but earlier study showed that *A. sylvestris* flowers are scarcely visited by moths or other night-active insects. Differences in quantitative scent composition observed also for other Apiaceae, e.g. *Laserpitium latifolium* (Borg-Karlson *et al.*, 1994), can likewise be related to non-pollinating insect visitors such as herbivores. This, however, requires further experimental study. It is unlikely, however, that the observed differences in floral phenotypes could be solely attributed to genetic drift because, given the size of populations and dependency of *A. sylvestris* reproduction on pollination and subsequent seed set, even weak selection could override the effects of random drift (Lande, 1976). Therefore, the overall picture appears to be the one of 'adaptive wandering', as described by Wilson and Thomson (1996). In this process, allopatric populations may diverge in response to local pollinator communities. This, however, does not result in a pollinator shift in *A. sylvestris* because the direction, strength and manner of selective pressure act 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 that seem to appeal to a wide spectrum of floral visitors. 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 adaptations for enhancing successful pollination included subtle modifications of floral traits (such as a corolla tube formed by folding of the petals and stamens; Lindsey and Bell, 1985).

In general, umbellifers, especially *A. sylvestris*, appear to be morphologically well adapted to ecological generalization (Lindsey and Bell, 1985; Corbet, 2006). Specialization in this plant family, perhaps, occurs only under very special circumstances, for example when the pollinator community is composed of insect visitor groups that clearly differ in their capacity to pollinate (e.g. due to differences in their functional morphology) and/or have different perceptual biases (e.g. for colour or scent). However, the barrier to the evolution of any morphological adaptation resulting in the fine-tuning of the flower towards particular pollinator types may arise from the architectural constraints on the floral bauplan that make umbellifers so uniform in their floral displays, and so successful in attracting large numbers of pollinators.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Fig. A: arrangement of potted plants during transplantation experiments. Fig. B:

inflorescence penetration ratio (proportion of visited umbellets in an umbel) by various visitor morphogroups for three study populations over the 3 years of study. Results of Kruskal–Wallis ANOVA are shown. Error bars indicate standard deviation of the mean. Vesp, wasps; Bee, bees; Syrph, hoverflies; Musc, muscoid flies; Col, beetles. Fig. C: *Angelica sylvestris* nectar sugar composition in the surveyed populations, expressed as percentages of fructose (Fru), glucose (Glu) and sucrose (Suc) relative to total sugars. Table S1: body pollen loads of insect visitors to *Angelica sylvestris* umbels in the three study populations. Table S2: scent emission of *Angelica sylvestris* in the three populations.

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