# Pollination biology and reproductive success of *Epipactis helleborine* (L.) Crantz (Orchidaceae) in anthropogenic and natural habitats

Agnieszka Rewicz, Radomir Jaskuła, Tomasz Rewicz, Grzegorz Tończyk

Background. *Epipactis helleborine* is an Eurasian species which prefers shaded woodland environments but it may also spontaneously and successfully colonise human-made artificial and disturbed habitats such as towns, parks and gardens. As it is suggested that orchids colonizing anthropogenic habitats are characterized by a specific set of features (eg. large plant size, fast flowers production) we wanted to compare pollination biology and reproductive success of *E. helloborine* from different habitat types. Methods. Pollination biology, reproductive success and autogamy in populations of *E. helleborine* from anthropogenic (roadside) and natural (mixed forest) habitats were compared. Eight populations (4 natural and 4 human-disturbed) in two seasons were studied according to height of plants, length of the inflorescence, as well as numbers of juvenile shoots, flowering shoots, flowers, and fruits. Number and diversity of insect pollinators, were studied in one natural and two human-disturbed populations. Results. Reproductive success (the ratio of the number of flowers to the number of seed sets (fruits)) in anthropogenic populations was significantly higher than in natural populations. In both types of populations the main insect pollinators were Syrphidae, Culicidae, Vespidae, Apidae and Formicidae. According to the type of pollinators' mouthparts, those with chewing (39%), sponging (34%) and chewing-sucking (20%) ones prevailed in anthropogenic habitats. In natural habitats pollinators with sponging (55%) and chewing mouthparts (32%) dominated, while chewing-sucking and piercing and sucking insects made respectively 9% and 4%. Discussion**.** We suggest that higher reproductive success of *E. helleborine* in anthropogenic than in natural populations may result from higher number of visits by pollinators and their greater species diversity but also from the bigger size of plants growing in such habitats. Moreover, our data clearly show that *E. helleborine* is an opportunistic species with respect to pollinators, with much wider spectrum of pollinating insects than it was suggested before.

# Pollination biology and reproductive success of *Epipactis helleborine* (L.) Crantz (Orchidaceae) in anthropogenic and natural habitats

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**Abstract**

**Background.** *Epipactis helleborine* is an Eurasian species which prefers shaded woodland environments but it may also spontaneously and successfully colonise human-made artificial and disturbed habitats such as towns, parks and gardens. As it is suggested that orchids colonizing anthropogenic habitats are characterized by a specific set of features (eg. large plant size, fast flowers production) we wanted to compare pollination biology and reproductive success of *E. helloborine* from different habitat types.

**Methods.** Pollination biology, reproductive success and autogamy in populations of *E. helleborine* from anthropogenic (roadside) and natural (mixed forest) habitats were compared. Eight populations (4 natural and 4 human-disturbed) in two seasons were studied according to height of plants, length of the inflorescence, as well as numbers of juvenile shoots, flowering shoots, flowers, and fruits.  Number and diversity of insect pollinators, were studied in one natural and two human-disturbed populations.

**Results.** Reproductive success (the ratio of the number of flowers to the number of seed sets (fruits)) in anthropogenic populations was significantly higher than in natural populations. In both types of populations the main insect pollinators were Syrphidae, Culicidae, Vespidae, Apidae and Formicidae. According to the type of pollinators' mouthparts, those with chewing (39%), sponging (34%) and chewing-sucking (20%) ones prevailed in anthropogenic habitats. In natural habitats pollinators with sponging (55%) and chewing mouthparts (32%) dominated, while chewing-sucking and piercing and sucking insects made respectively 9% and 4%.

**Discussion.** We suggest that higher reproductive success of *E. helleborine* in anthropogenic than in natural populations may result from higher number of visits by pollinators and their greater species diversity but also from the bigger size of plants growing in such habitats. Moreover, our

data clearly show that *E. helleborine* is an opportunistic species with respect to pollinators, with much wider spectrum of pollinating insects than it was suggested before.

**Key words**: apophytes, autogamy, anthropogenic habitats, natural habitats

## Introduction

Orchidaceae is the most diverse plant family (20 000 - 30 000 species) (Bauman, Kunkele & Lorenz, 2010; Djordjević et al., 2014) with many species that are seriously endangered and require conservation efforts to maintain their populations. On the other side we observe appearance of numerous orchid species in anthropogenic habitats (Dickson, 1990; Light & MacConail, 1991, 2005, 2006; Hollingsworth & Dickson, 1997). The most common colonizers of such secondary habitats in the Palearctic are members of the genera *Epipactis* and *Dactylorhiza*, characterized by short life cycles and broad ecological niches (Adamowski, 2004, 2006; Esfeld et al., 2008).

*Epipactis helleborine* (broad-leaved helleborine) is an Eurasian species (Delforge, 2006) introduced in the XIX century (Owen, 1879) to several regions of North America (Procházka & Velísek 1983). It prefers shaded woodland environments and its nodding flowers vary in colour from greenish pink to purple. Moreover, this species occurs in wide spectrum of habitats and often acts as a pioneer in restated areas (Piękoś-Mirkowa & Mirek, 2006). It may also appear spontaneously in urban areas such as towns, parks and gardens (Nikolaeva & Zefirov, 1971; Dickson, 1990). Frequently its populations are found around limestone quarries and mine dumps (Świercz, 2004, 2006; Kiedrzyński & Stefaniak, 2011). *Epipactis helleborine* is thus a fine example of apophytism - i.e. a native species growing in disturbed or human-made habitats

(Adamowski & Conti, 1991; Hollingsworth & Dickson, 1997; Sukopp, 2006; Rewicz et al., 2015, Rewicz, Kołodziejek, Jakubska-Busse, 2016). Forman et al. (2009) suggested that orchids colonizing anthropogenic habitats are characterized by a specific set of features: fast growth resulting in large plant size, fast flowers production and light anemochoric seeds. *Epipactis helleborine* may produce seeds in allogamy and optionally, in autogamy (Tałałaj & Brzosko 2008).

Important aspect of orchid population biology is a reproductive system (Machaka-Houri, et al. 2012). Seeds produced by orchids are very specific and due to their extremely small sizes, called "seed dust" (Arditti, Michaud & Healey, 1979; Arditti, Michaud & Healey, 1980; Rasmussen & Whigham, 2002; Tałałaj & Brzosko, 2008). *Epipactis helleborine* may produce from 1000 to 2200 seeds in one fruit (bag) (Arditti & Ghani, 2000; Rewicz, Kołodziejek & Jakubska-Busse, 2016). High fecundity of orchids results of the large number of seeds with much reduced weight (from 0.31 μg to 24 μg, depending on species) (Arditti, 1967). However, the high number of seeds does not lead to high recruitment of seedlings (Brzosko, 2000). It may results from specific biology of orchids, i.e. obligatory presence of mycorrhizal symbionts during germination and further plant growth (Szlachetko, 1995). Low reproduction success (the ratio of the number of flowers to the number of seed sets (fruits) (Doust & Doust, 1988)) may also arise from high level of morphological adaptation of flowers to particular pollinators. Some orchid species developed specific mechanisms such as: flower traps in *Cypripedium calceolus* or sexual deception and pseudo-copulation in *Ophrys* spp. Human disturbance as habitat transformation is regarded as a principal cause of  pollinator decline in global scale (Goulson et al., 2008). Orchids are even more prone to that adverse trend , because up to 70% of species are pollinated by particular species of pollinator (Neiland & Wilcock, 1998). Deficiency of suitable pollinators may also be a reason of low reproductive success. Thus, autogamy is an alternative way of seeds production. A question arises which reproductive system is preferred by orchids rapidly and successfully colonizing anthropogenic habitats (Light & MacConnail, 2006). *Epipactis helleborine* can be a suitable model species, as it occurs in both natural and disturbed habitats. Knowledge of the diversity of pollinators of this species can help in assessing the ability to quick adaptation to different disturbed habitats in which it occurs.

In this study we wanted to find the differences (if any) in reproductive success between both natural and anthropogenic habitats and what might explain those differences. Specifically, we addressed the following questions: a) what is the composition of pollinator fauna of *E. helleborine* in anthropogenic and natural habitats, b) does the number of capsules produced through autogamy and natural pollination differ between anthropogenic and natural populations, c) is the reproductive success of *E. helleborine* different in populations from anthropogenic and natural populations?

**Materials and methods**

***Reproductive success***

We accepted definition of reproductive success after Doust & Doust, (1988) as: the ratio of the number of flowers to the number of seed sets (fruits). Eight populations of *E. helleborine* were studied in two seasons, 2011 and 2012. The identified habitat types were separated in two categories. One included human-disturbed habitats, such as roadsides (population A1 – between the road and a wooden fence in a Guszczewina village, A2 – close to the car parking in Hajnówka, A3 – in the thicket by the roadside in Sulejów, A4 – on a roadside bordering pine forest in Sulejów). The other one grouped natural habitats (population N1 - in the forest *Galio* *sylvatici-Carpinetum betuli* Oberd. 1957 in Kotowice, N2 – in the forest *Galio-Carpinetum* Oberd. 1957 in Kaczawskie Mts., N3, N4 – *Galio-Carpinetum* Oberd. 1957 in the Strict Reserve Bialowieża Primaeval Forest (Fig. 1). All shoots found in each population were measured (Tab. 1). The following parameters were recorded for each plant in a population: number of juvenile shoots (JS), number of flowering shoots (NFS), number of flowers (NF), number of fruits (capsules) (NFR), height of plants (HP), length of the inflorescence (LI). Density of each population was also measured as [shoots/m2].

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***Flower visitors***

Pollinators were both caught and observed in two anthropogenic populations of *E. helleborine*: roadside in Guszczewina village (A1), and close to the car park in Hajnówka city (A2), and in one natural population in the Białowieża Primeval Forest (N3). All these populations were located in eastern Poland (Fig 1.).

Insects were caught using entomological hand nets from the fresh orchid flowers in two terms: 15-23.07.2011 and 13-22.07.2012. The fieldwork was carried out during days with sunny weather. Air temperature was measured every two hours with Volkraft thermometer. In each *E. helloborine* population, the insects were caught by two-people teams between 9 a.m and 7 p.m. Insects were collected from 10 shoots growing close to each other. Material was collected until the transfer of pollinia by insects was observed. Insects were killed using ethyl acetate and preserved in 75% ethanol (except bumble-bees *Bombus* spp. which are protected by Polish law – these specimens were only photographed). Pollinators we recognized as insects which arrived with attached pollinia, or departed the flower with pollinia. All the insects were identified to family level. The most common insects in the populations were identified to species level. Moreover, as many insect species can collect nectar/pollen only from one-few morphological types of flowers, we categorized pollinators with their mouth-part types.

***The ability of E. helleborine to autogamy***

The experiment was carried out from July to September 2012. Ten shoots in early stage of flowering (closed buds) were selected in each population. Flowers on each shoot were counted and the inflorescence was covered by bags made from mosquito net. After three months the isolators were removed and the number of fruit sets was counted (Fig. 2, Tab. 1). Viability of seed was examined by tetrazolium test (live seed with stained embryos and dead seed with unstained embryos) (Van Waes & Debergh 1986).

***Data analysis***

The software package STATISTICA PL. ver. 10 (Stat-Soft Inc., 2011) was used for all statistical analyses (van Emden, 2008). To compare the number of fruits (capsules) produced by autogamy in different habitats we used the Mann-Whitney U-test. To compare the reproductive success between habitats we used the Student's *t*-test. Diversity of pollinator fauna between natural and anthropogenic habitats was evaluated using chi-squared test. Correlation between the number of flowers and number of fruits in inflorescence in different habitats was evaluated using the Spearman’s correlation coefficient (Meissner, 2010).

## Results

***Flower visitors***

Pollinators of *Epipactis helleborine* collected during this study belonged to six orders and 23 families of insects (Tab. 2). In case of anthropogenic populations the taxonomic diversity of pollinators was higher, with 19 families grouped in five orders, while in the Białowieża Primeval Forest, we noted only 14 families from four orders (statistically significant values, chi-squared test, p = 0.00). In both types of habitats, Diptera and Hymenoptera clearly predominated, respectively 41% and 52% of all the pollinators observed in anthropogenic environments, and 59% and 37% observed in then Białowieża Primeval Forest (Fig. 4, 5). Coleoptera were the third main group of pollinators making up 6% in anthropogenic and 4% in natural populations. Occasionally, single individuals of grasshoppers (Orthoptera), earwigs (Dermaptera) and scorpion flies (Mecoptera) were also noted as pollinators of *E. helleborine*.

In anthropogenic populations, the main dipteran pollinators were hoverflies (Syrphidae) making up 63% of dipteran pollinators and 26% of all the observed pollinators – with the most frequent species *Meliscaeva cinctella* and *Episyrphus balteatus*, followed by mosquitoes (Culicidae) (18% of dipteran and 7% of all pollinators). The main hymenopteran pollinators were wasps (Vespidae – 42% of hymenopteran and 22% of all pollinators – with the most frequent species *Dolichovespula saxonica*), bees (Apidae – 38% and 20%, respectively, with the main pollinator *Apis mellifera*), and ants (Formicidae) (16% and 8% respectively). In the Białowieża Primeval Forest, true flies (Syrphidae) made 90% (53% of all; with *Meliscaeva cinctella* as most frequent species), and mosquitoes (Culicidae) made 7% of pollinators (4% of all), while the main hymenopteran pollinators were wasps (Vespidae) (58% of hymenopterans and 21% of all pollinators; with *Dolichovespula saxonica* as most frequent pollinator), bees (Apidae) (24% and 9%, respectively), and ants (Formicidae) (17% and 6%, respectively).

According to the type of mouthparts, the pollinators of *E. helleborine* can be ascribed to four groups: 1/ sponging insects (Diptera excluding Culicidae) (44% of all noted pollinators), 2/ chewing (= mandibulate) insects (Hymenoptera excluding Apidae, Coleoptera, Dermaptera, Orthoptera and Mecoptera) (36%), 3/ chewing-sucking insects (Apidae) (14%), and 4/ piercing and sucking insects (Culicidae) (6%) (Fig. 5).

In the Białowieża Primeval Forest the main groups of pollinators of *E. helleborine* were, sponging (55%) and chewing insects (32%), while the chewing-sucking and piercing and sucking insects made respectively 9% and 4% of all pollinators. In the anthropogenic populations, the most frequent pollinators belonged to chewing (39%), sponging (34%) and chewing-sucking (20%) insects, and only 7% of the noted insects were characterized by piercing and sucking mouthparts.

***Autogamy***

Mean number of capsules (20) produced in autogamy in anthropogenic populations was significantly higher than the number of capsules produced in Białowieża Primeval Forest (12 individuals) (U Mann-Whitney test, $p = 0.0008$, $p < 0.05$). Both in the natural and anthropogenic populations, the number of capsules was strongly positively correlated (respectively $r = 88$ and $r = 98$, $p < 0.05$) with the number of flowers in inflorescence (Tab. 3). In case of natural pollination, in both anthropogenic and natural populations, the number of fruits was the same (19).

In Białowieża Primeval Forest, the number of fruits produced in by open - pollination was slightly higher than the number of fruits produced by autogamy, however the difference was not significant (U Mann-Whitney test, $p = 0.48$, $p < 0.05$). Amount of dead seeds (with unstained embryo) resulting from autogamy varied from 70.5% to 75.4%, and were higher comparing to natural pollination (varied from 48.8% to 50.3%) (Tab. 4).

***Reproductive success***

Reproductive success in anthropogenic populations (87.1%) was significantly higher than in natural populations (72.3%) (Student's *t*-test, p=0.0008, $p < 0.05$) (Fig. 6). In anthropogenic populations it ranged from 77.8% (A4) to 100% (A2) while in the natural ones it ranged from 44.4% (N1) to 83.3% (N4) (Tab. 5). Number of flowers in natural populations ranged from 20 (in 2011) to 22 (in 2012), while in anthropogenic populations – from 15 (2011) do 14 (2012).

The strongest correlation was found between the reproductive success and height of plants ($r = 0.82$, $p < 0.05$) in anthropogenic populations. No significant correlation was found between the reproductive success and population density. In natural populations a weak correlation was found between reproductive success and density of populations ($r = 0.40$, $p < 0.05$). Regression analysis was significant, only in case of anthropogenic populations, between reproductive success and height of plants as well as between reproductive success and number of flowers (Fig. 6).

## Discussion

***Pollinators***

Traditionally, the orchid *Epipactis helleborine* is demonstrating different morphological and physiological adaptations to attract social wasps as pollinators (eg. Müller, 1873; Darwin, 1877; van der Pijl & Dodson, 1966; Judd,1971, 1979; Müller, 1988; van der Cingel, 1995; Delforge, 2006; Claessens & Kleynen 2011; Charles, 2012). According to literature its main pollinators are wasps belonging to the following genera: *Vespula* (*V. vulgaris* and *V. germanica),* *Vespa* (*V. sylvestris* and *V. vulgaris*), and *Dolichovespula* (*D. saxonica, D. sylvestris* and *D.*

*media)* (Claessens & Kleynen 2011). On the other hand, at least in some regions of the orchid distributional range additional insect groups such as flies and beetles may play an important role in pollination (Jakubska et al., 2005a, b; Fateryga, 2012; Claessens & Kleynen 2014). As shown above, pollinators of *E. helleborine* noted during our studies belonged to six orders and 24 insect families (Fig. 4). All these insects could be characterized by four different types of mouthparts adapted to collect food in different ways (Fig. 5). Similar results were noted by Jakubska et al. (2005a) who observed five coleopteran, four hymenopteran, two dipteran, and one lepidopteran family acting as pollinators of this orchid species and also belonging to four groups according to type of mouthparts. In comparison to our results, only piercing and sucking insects were not recorded as pollinators of *E. helleborine* by the above authors while, on the other hand, they noted sucking insects (Lepidoptera) which we did not observed. Such high taxonomical diversity of insects as well as their diverse morphological adaptations of mouthparts (all five main types of insect mouthparts) used for collecting nectar and pollen, clearly suggests that *E. helloborine* is much more opportunistic species according to pollinators than it was suggested before. As it was shown by Jacquemyn et al. (2014), such strategy can be used also in some other species belonging to this orchid genus. In their summary of knowledge upon pollinators of *E. palustris*, the authors noted members of six families of Coleoptera, 22 of Diptera, 12 of Hymenoptera, and one of Heteroptera (wrongly placed among Hymenoptera). On the other hand it seems that a few insect groups play much more important role in pollination biology of *E. helleborine* that the others. In our studies, both in natural and anthropogenic sites, sponging (flies, mainly Syrphidae) and chewing insects (mainly Vespidae and Formicidae but also Coleoptera) dominated, with the chewing-sucking insects (Apidae) as the third group according to frequency (Tab. 2, Fig. 4, 5). Such differences may result from: geographical localization, taxonomy of pollinators fauna, weather conditions and especially air temperature, which may change emission of attractants contained in nectar of *Epipactis* (Ehlers & Olesen, 1997). The important role of Vespidae as well as of Syrphidae and Apidae was observed not only in other Polish populations of *E. helleborine* (Jakubska et al., 2005a, b), but also in other regions of Europe (eg. Fateryga, 2012; Claessens & Kleynen, 2014). It is also known for another species of *Epipactis* like: *E. palustris* (eg. Nilsson, 1978; Brantjes, 1981; Verbeke & Verschueren 1984; Vöth, 1988; Jakubska-Busse & Kadej, 2008; Fateryga, 2012; Jacquemyn, Brys & Hutchings, 2014), *E. atrorubens* (Jakubska-Busse & Kadej, 2011), *E. consimilis* (Ivri & Dafni, 1977), *E. turcica* (Fateryga, 2012) and *E. veratrifolia* (Jin et al., 2014). As a result, at least some insect species visit *Epipactis* not only for its highly energetic pollen and/or nectar but also to look for prey i.e. other insects attracted by flower (Rico-Gray & Oliveira, 2007). For example Vespidae, Crabronidae or Ichneumonidae, but also some Syrphidae flies, can be classified as such predators. As it was shown by Turlings, Tumlinson & Lewis (1990) and Brodmann et al. (2008) for some Vespidae wasps, at least some *Epipactis* species (including *E. helleborine* and *E. purpurata*) are producing green-leaf volatiles (GLVs), whose chemical composition is very similar to those emitted by damaged plant tissues when the plant is attacked by caterpillars. The latter are known as one of the most important prey for wasps. Surprisingly Jin et al. (2014) noted that females of some Syrphidae can lay their eggs on orchid attacked by aphids (Aphidoidea) which are main food for their hatched larvae. The explanation of this phenomenon was provided by Stökl et al. (2010) who noted that flowers of *E. veratrifolia* are visited by some aphidophagous Syrhipidae as the orchid produces a- and b-pinene, b-myrcene and b-phellandrene. These substances are very similar to aphid-derived kairomones, which normally are emitted as alarm pheromones by several aphid species. Hoverflies were also noted as important pollinators of *E. helleborine* both during our studies

(Tab. 2, Fig. 4) and by Jakubska et al. (2005a). Moreover, aphids are regularly noted as feeding on this orchid species (pers. observations). Thus, we can suppose that such chemical mimicry is more common among *Epipactis* species.

Pollinator availability is one of the key aspects of the reproductive success of orchids. This is all the more urgent problem because about 70% of species is closely related to the specific pollinator species (Neiland & Wilcock, 1998). Pollinator diversity decline is mainly due to the transformation of the environment associated with urbanization, agricultural development and transformation of the area. Numerous studies have confirmed declining, diversity and changes in species composition bees and hoverflies in cities and disturbed areas (Banaszak - Cibicka & Żmihorski, 2012; Schweiger et al., 2007). Our results indicate that *Epipactis helleborine* has a diverse group of pollinators which promotes the genre in a very rapidly changing areas transformed by man and one of the key features apophytes.

***Reproductive success and effect of autogamy of E. helleborine seeds***

Autogamy in *E. helleborine* was observed by many authors, some of them (Darwin, 1877; Weijer, 1952; Richards & Porter, 1982; Robatsch, 1983) claimed that this species shows optional autogamy (mixed-mating ) (Ehlers & Pedersen, 2000; Claessens & Kleynen, 2011). However, Ehlers et al. (2002) suggested that autogamy in *E. helleborine* is rare and that this phenomenon occurs only in strict conditions, i.e. when suitable pollinators are lacking. Our results proved, that autogamy occurs in both anthropogenic and natural populations of *E. helleborine* (Tab. 3). Despite some differences between number of fruits in populations from both habitats, we found no significant differences between number of fruits formed by autogamy and by natural pollination, which is congruent with work of Weijer (1952). In our opinion autogamy is a common phenomenon in life cycle of *E. helleborine*. Fruits were produced in both types of habitats.

On the other hand, reproductive success of *E. helleborine* was higher in anthropogenic habitats (87.1%) than in the natural ones (72.3%). We suggest that it may be caused by higher number of visits and a greater species diversity of pollinators as well as by larger size of plants. Higher plants from anthropogenic habitats are more accessible for insects, which influence greater reproductive success. The relationship between plant height and reproductive success was confirmed by Machaka-Houri et al. (2012) in their studies upon *Orchis galilea*. Our results suggest also that there is no association between reproductive success and number of flowers on a sprout both in anthropogenic and natural populations. It appears that in orchids, height of the plant and number of flowers matters enhances attractiveness for insect pollinators (Kindlmann & Jersakova, 2005). Specimens from anthropogenic populations presented in our study were higher, had bigger flowers and more diverse pollinating fauna. Plants with bigger size of shoots and more flowers are more tempting for pollinators, which results in more efficient transport of pollinia (van der Piper & Waite, 1988). According to Janzen (1971) as well as Light and MacConaill (1998) simultaneous opening of flowers in anthropogenic populations increases number of pollinators' visits and pollinia transport. Our results confirm these theses. Moreover, no significant correlation between density of plants in anthropogenic populations was noted and in natural populations such correlation was weak. Similar results were obtained in other studies (Sih & Baltus, 1987; Agren, 1989; Alexanderson & Agren, 1996; Ehlers, Olesen & Gren, 2002).

In conclusion, our research demonstrates that spectrum of insects pollinating *E. helleborine* is much wider than it was suggested in literature. Increased variety of possible pollinators allows faster and better adaptation to the human-changed environment. Reproductive success of *E. helleborine* was higher in anthropogenic habitats and it may be caused by higher number of visits and a greater species diversity of pollinators as well as by larger size of plants. Moreover autogamy is not uncommon in reproduction strategy, and we found no significant differences between number of fruits formed by autogamy and by natural pollination.

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## References

Adamowski W. 2004. Population expansion of native orchids in anthropogenous habitats. *Neobiota* 3: 86.

Adamowski W. 2006. Expansion in native orchids in anthropogenous habitats. *Polish Botanical Studies* 22: 35–44.

Adamowski W, Conti F. 1991. Mass occurrence of orchids in poplar plantations near Czeremcha village as an example of apophityzm. *Phytocenosis* 3: 259–267.

Agren J. 1989. Seed size and number in *Rubus chamaemorus*: between habitat variation, and effects of defoliation and supplemental pollination. *Journal of Ecology* 77: 1080–1092 doi: 10.2307/2260824

Alexanderson R, Agren J. 1996. Population size, pollinator visitation rate and fruit production in the deceptive orchid *Calypso bulbosa*. *Oecologia* 107: 533–540 doi: 10.1007/BF00333945

Arditti J. 1967. Factors affecting the germination of orchid seeds. *Botanical Review* 33: 1–97.

Arditti J, Ghani AKA. 2000. Numerical and physical properties of orchid seeds and their biological implications. *New Phytologist* 145: 367–421 doi: 10.1046/j.1469-8137.2000.00587.x

Arditti J, Michaud JD, Healey PL. 1979. Morphometry of orchid seeds: I. *Paphiopedilum* and native California and related species of *Cypripedium*. *American Journal of Botany* 66(10): 1128–1137 doi: 10.2307/2442211

Arditti J, Michaud JD, Healey PL. 1980. Morphometry of orchid seeds II. Native California and related species of *Calypso, Cephalanthera, Corallorhiza* and *Epipactis*. *American Journal of Botany* 67(3): 347–360 doi: 10.2307/2442345

Banaszak-Cibicka W, Żmihorski M. 2012. Wild bees along an urban gradient: winners and losers. *Journal of Insect Conservation* 16: 331-343 doi: 10.1007/s10841-011-9419-2

Baumann H, Kunkele S, Lorenz R. 2010. *Storczyki Europy i obszarów sąsiednich*. Multico, Warszawa.

Brantjes NBM. 1981. Ant, bee and fly pollination in *Epipactis palustris* (L.) Crantz (Orchidaceae). *Acta Botanica Neerlandica* 30:59–68 doi: 10.1111/j.1438-8677.1981.tb00387.x

Brodmann J, Twele R, Francke W, Hölzler G, Zhang QH, Ayasse M. 2008. Orchids Mimic Green-Leaf Volatiles to Attract Prey-Hunting Wasps for Pollination. *Current Biology* 18: 740–744 doi: 10.1016/j.cub.2008.04.040

Brzosko E. 2000. Zmiany liczebności populacji roślin o różnych strategiach reprodukcyjnych w procesie sukcesji. *Wiadomości Botaniczne* 44: 13–22.

Claessens J, Kleynen J. 2011. *The flower of the European orchid. Form and function*. Schrijen-Lippertz, Netherlands.

Claessens J, Kleynen J. 2014. The pollination of European Orchids Part 3: *Limodorum* and *Epipactis*. *Journal of the Hardy Orchid Society* 11 (2): 64–71.

Charles LA. 2012. *The pollination biology of North American Orchids: Volume 2*. Springer New York, Dordrecht, Heidelberg, London.

Darwin C. 1877. *The various contrivances by which orchids are fertilised by insects*. London doi: 10.5962/bhl.title.60877

Delforge P. 2006. *Orchids of Europe, North Africa and the Middle East*. Timber Press Inc., Oregon, USA.

Dickson JH. 1990. *Epipactis helleborine* in gardens and other urban habitats: an example for apophytism. In: Sukopp H, Hejny S(eds) *Urban ecology*. SPB Academic Publishing, The Hague, pp 245–249.

Djordjević V, Tsiftsis S, Lakušić D, Stevanović V. 2014. Niche analysis of orchids of serpentine and non-serpentine areas: Implications for conservation. *Plant Biosystems* 10 pp doi: 10.1080/11263504.2014.990534

Doust JL, Doust L. 1988. P*lant reproductive ecology: patterns and strategies*. New York. Oxford University Press.

Ehlers BK, Olesen JM. 1997. The fruit-wasp route to toxic nectar in *Epipactis* orchids? *Flora* 192: 223–229.

Ehlers BK, Pedersen H. 2000. Genetic variation in three species of *Epipactis* (Orchidaceae): geographic scale and evolutionary inferences. *Biological Journal of the Linnean Society* 69: 411–430 doi: 10.1111/j.1095-8312.2000.tb01214.x

Ehlers BK, Olesen JM, Gren JA. 2002. Floral morphology and reproductive success in the orchid *Epipactis helleborine*: regional and local across-habitat variation. *Plant Systematic and Evolution* 236(1/2): 19–32 doi: 10.1007/s00606-002-0197-x

Esfeld K, Hensen I, Wesche K, Jakob SS, Tischew S, Blattner FR. 2008. Molecular data indicate multiple colonizations of former lignite mining areas in Eastern Germany by *Epipactus palustris* (Orchidaceae). *Biodiversity and Conservation* 17:2441–2453 doi: 10.1007/s10531-008-9391-7

Fateryga AV. 2012. Pollination ecology of the species from the genus *Epipactis* (Orchidaceae) in the Crimea. *Ekosistemy, ikh Optimizatziya i Okhrana* 6: 136–150.

Forman RTT, Sperling D, Bissonette J, Clevenger AP, Cutshall C, Dale V, Fahrig L, France R, Goldman C, Heanue K, Jones J, Swanson F, Turrentine T, Winter T. 2009. *Road ecology*. Science and solutions. Island Press, Washington, USA

Goulson D, Lye GC, Darvill B. 2008. Decline and conservation of bumble bees. *Annual Review of Entomology* 53:191–208 doi: 10.1146/annurev.ento.53.103106.093454

Hollingsworth PM, Dickson JH. 1997. Genetic variation in rural and urban populations of *Epipactis helleborine* (L.) Crantz. (Orchidaceae) in Britain. *Botanical Journal of the Linnean Society* 123: 321–331 doi: 10.1111/j.1095-8339.1997.tb01422.x

Ivri Y, Dafni A. 1977. The pollination ecology of *Epipactis consimilis* Don (Orchidaceae) in Israel. *New Phytologist* 79: 173–177 doi: 10.1111/j.1469-8137.1977.tb02193.x

Jacquemyn H, Brys R, Hutchings MJ. 2014. Biological Flora of the British Isles: *Epipactis palustris*. *Journal of Ecology* 102:1341–1355 doi: 10.1111/1365-2745.12287

Jakubska A, Kadej M, Prządo D, Steininger M. 2005a. Pollination ecology of *Epipactis helleborine* (L.) Crantz (Orchidaceae, Neottieae) in the south-western Poland. *Acta Botanica Silesiaca* 2: 131–144.

Jakubska A, Kadej M, Prządo D, Steininger M. 2005b. How do orchids lure pollinators? Nectar chemical composition of *Epipactis helleborine* s.l. (Orchidaceae, Neottieae) and its influence on insects reactions in field conditions. *Pesticides* 4: 175–180.

Jakubska A, Prządo D, Steininger M, Anioł-Kwiatkowska J, Kadej M. 2005c. Why do pollinators become "sluggish"? Nectar chemical constituents from *Epipactis helleborine* (L.) Crantz (Orchidaceae). *Applied Ecology and Environmental Research* 3(2): 29-38 doi: 10.15666/aeer/0302_029038

Jakubska-Busse A, Kadej M. 2008. Pollination ecology of Marsh Helleborine *Epipactis palustris* (L.) Crantz on the Polish side of the Orlickie Mts. (Central Sudety Mts.). In: Kočárek P, Plášek V, Malachová K, Cimalová Š (eds) *Environmental changes and biological assessment IV*. Scripta Facultatis Rerum Naturalium Universitatis Ostraviensis, Ostrava pp. 247–252.

Jakubska-Busse A, Kadej M. 2011. The pollination of *Epipactis* Zinn, 1757 (Orchidaceae) species in central Europe – the significance of chemical attractants, floral morphology and concomitant insects. *Acta Societatis Botonicorum Poloniae* 80: 49–57 doi: 10.5586/asbp.2011.007

Janzen D. 1971. Euglossine bees as long-distance pollinators of tropical plants. *Science* 171: 203–205 doi: 10.1126/science.171.3967.203

Jin XH, Ren ZX, Xu SZ, Wang H, Li DZ, Li ZY. 2014. The evolution of floral deception in *Epipactis veratrifolia* (Orchidaceae): from indirect defense to pollination. *BMC Plant Biology* 14(1): 63 doi: 10.1186/1471-2229-14-63

Judd WW. 1971. Wasps (Vespoidea) pollinating Helleborine *Epipactis helleborine* (L.) Crantz, at Owen Sound, Ontario. *Proceedings of the Entomological Society of Ontario* 102:115–118.

Judd WW. 1979. Arthropods associated with Helleborine Orchid, *Epipactis helleborine*. *Entomological News* 90(1): 41–44.

Kiedrzyński M, Stefaniak A. 2011. Występowanie rodzaju *Epipactis* Zinn 1757 w Puszczy Pilickiej na stanowiskach naturalnych i antropogenicznych. In: Brzosko E, Wróblewska A, Jarmakowicz E (eds) *Storczykowate w Polsce. Biologia i Ochrona.* Białystok, pp 86–96.

Kindlmann P, Jersakova J. 2005. Floral display, reproductive success, and conservation of terrestrial orchids. *Selbyana* 26: 136–144.

Light MHS, MacConaill M. 1991. Patterns of appearance in *Epipactis helleborine* (L.) Crantz. In: Wells T.C.E., Willems J.H (eds) *Population ecology of terrestrial orchids*. Academic, The Hague, The Netherlands, pp 77–87.

Light MHS, MacConaill M. 1998. Factors affecting germinable seed yield in *Cypripedium calceolus* var. *pubescens* (Willd.) Correll and *Epipactis helleborine* (L.) Crantz (Orchidaceae). *Botanican Journal of the Linnean Society* 126: 3–26 doi: 10.1111/j.1095-8339.1998.tb02512.x

Light MHS, MacConaill M. 2005. Long term studies – case for orchid species survival. *Selbyana* 26: 174–188.

Light MHS, MacConaill M. 2006. Appearance and disappearance of a weedy orchid, *Epipactis helleborine*. *Folia Geobotanica* 41: 77–93 doi: 10.1007/BF02805263

Machaka-Houri N, Al-Zein MS, Westbury DB, Talhouk SN. 2012. Reproductive success of the rare endemic *Orchis galilaea* (Orchidaceae) in Lebanon. *Turkish Journal of Botany* 36: 677–682.

Meissner W. 2010. Przewodnik do ćwiczeń z przedmiotu metody statystyczne w biologii, Wydawnictwo Uniwersytetu Gdańskiego.

Müller H. 1873. *Die Befruchtung der Blumen durch Insekten und die gegenseitigen Anpassungen beider*. Leipzig, Germany: Engelmann Verlag doi: 10.5962/bhl.title.50246

Müller I. 1988. Vergleichende blütenökologische Untersuchungen an der Orchideengattung *Epipactis*. *Mitteilungen des Arbeitskreises Heimische Orchideen Baden-Württemberg*

20 (4): 701–803.

Neiland MM, Wilcock CC. 1998. Fruit set, nectar reward, and rarity in the Orchidaceae. *American Journal of Botany* 85: 1657–1671 doi: 10.2307/2446499

Nikolaeva VM, Zefirov BM. 1971. F*lora Belovezhskoi pushchi (sosudistye sporovye i semennye rasteniya)*. Minsk: Uradzhai

Nilsson LA. 1978. Pollination ecology of *Epipactis palustris* (Orchidaceae). *Botaniska Notiser*. 131: 255–368.

Owen ML. 1879. An orchid new to America. *Bulletin of the Torrey Botanical Club* 6: 329–330.

Piękoś-Mirkowa H, Mirek Z. 2006. *Atlas roślin chronionych*. Seria Flora Polski. Warszawa

Procházka F, Velísek V. 1983. *Orchideje nasi prirody*. Prague, Ceskoslovenska Akademie

Rasmussen HN, Whigham DF. 2002. Phenology of roots and mycorrhiza in five orchid species differing in phototrophic strategy. *New Phytologist* 154: 797–807 doi: 10.1046/j.1469-8137.2002.00422.x

Rewicz A, Zielińska KM, Kiedrzyński M, Kucharski L. 2015. Orchidaceae in the anthropogenic landscape of Central Poland: diversity, extinction and conservation perspectives. *Archives of Biological Sciences* 67: 119–130.

Rewicz A, Kołodziejek J, Jakubska-Busse A. 2016. The role of anthropogenic habitats as substitutes for natural habitats: a case study on *Epipactis helleborine* (L.) Crantz (Orchidaceae, Neottieae). Variations in size and nutrient composition of seeds. *Turkish Journal of Botany* doi:10.3906/bot-1404-69.

Richards AJ, Porter AF. 1982. On the identity of a Northumberland *Epipactis*. *Watsonia* 14: 121–128.

Rico-Gray V, Oliveira PS. 2007. *The Ecology and Evolution of Ant-Plant Interactions*. University of Chicago Press doi: 10.7208/chicago/9780226713540.001.0001

Robatsch K. 1983. Beiträge zur Blütenbiologie und Autogamie der Gattung *Epipactis. Jahresber Naturwiss Ver Wuppertal* 36: 25–32.

Schweiger O, Musche M, Bailey D, Billeter R, Diekötter T, Hendrickx F, Herzog F, Liira J, Maelfait JP, Speelmans M, Dziock F. 2007. Functional richness of local hoverfly communities (Diptera, Syrphidae) in response to land use across temperate Europe. *Oikos* 116: 461-472 doi: 10.1111/j.2007.0030-1299.15372.x

Sih A, Baltus M. 1987. Patch size, pollinator behavior and pollinator limitation in Catnip. *Ecology* 68: 1679–1690 doi: 10.2307/1939860

StatSoft Inc. S (2011) *STATISTICA (data analysis software system), version 10*. www.statsoft.com

Stökl J, Brodmann J, Dafni A, Ayasse M, Hansson BH. 2010. Smells like aphids: orchid flowers mimic aphid alarm pheromones to attract hoverflies for pollination. *Proceedings of the Royal Society B-Biological Science* doi:10.1098/rspb.2010.1770 doi: 10.1098/rspb.2010.1770

Sukopp H. 2006. Apophyts in the flora of Central Europe. *Polish Botanical Studies* 22: 473–485.

Szlachetko D. 1995. *Systema Orchidarium*. Szafer Institute of Botany, Kraków.

Świercz A. 2004. The cement and lime industry and the occurrence of selected species of orchids. *Przegląd Przyrodniczy* 15: 117–123.

Świercz A. 2006. Przegląd stanowisk wybranych gatunków z rodziny Orchideaceae w sąsiedztwie cementowni regionu świętokrzyskiego. Regionalne Studia Ekologiczno-Krajobrazowe. *Problemy Ekologii Krajobrazu* 16: 433–440.

Tałałaj I, Brzosko E. 2008. Selfing potential in *Epipactis palustris*, *E. helleborine* and *E. atrorubens* (Orchidaceae). *Plant Systematic and Evolution* 276: 21–29 doi: 10.1007/s00606-008-0082-3

Turlings TCJ, Tumlinson JH, Lewis WJ. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250: 1251–1253 doi: 10.1126/science.250.4985.1251

van Emden H. 2008. Statistics for terrified biologists. Blackwell Publishing, USA, UK, Australia.

van der Cingel NA. 1995. *An atlas of orchid pollination: European orchids*. Rotterdam: A. A. Balkema.

van der Pijl L, Dodson CH. 1966. *Orchid flowers: their pollination and evolution*. Miami, FL Fairchild Tropical Garden and the University of Miami Press.

van der Piper J, Waite S. 1988. The gender role of flowers of broad leaved Helleborine *Epipactis helleborine* (L.). *Functional Ecology* 2: 35–40 doi: 10.2307/2389457

Van Waes JM, Debergh PC. 1986. Adaptation of the tetrazolium method for testing the seed viability, and scanning electron microscopy study of some Western European orchids. *Physiol. Plant.* 66: 435–442.

Verbeke C, Verschueren Y. 1984. La pollinisation de l'Epipactis des marais (*Epipactis palustris*) du Bakkersdam (Pays-Bas). *Les Naturalistes Belges* 65: 175–192.

Vöth W. 1988. Die Honigbiene, *Apis mellifera*, L. (Apidae), erwiest sich als der effektive Bestaüber von *Epipactis palustris* (L.) Crantz. *Mitteilungen des Arbeitskreises Heimische Orchideen Baden-Württemberg* 19: 112–118.

Weijer J. 1952. The colour differences in *Epipactis helleborine* (L.) Cr. Wats. & Coult., and the selection of the genetical varieties by environment. *Genetica* 26: 1–32 doi: 10.1007/BF01690613

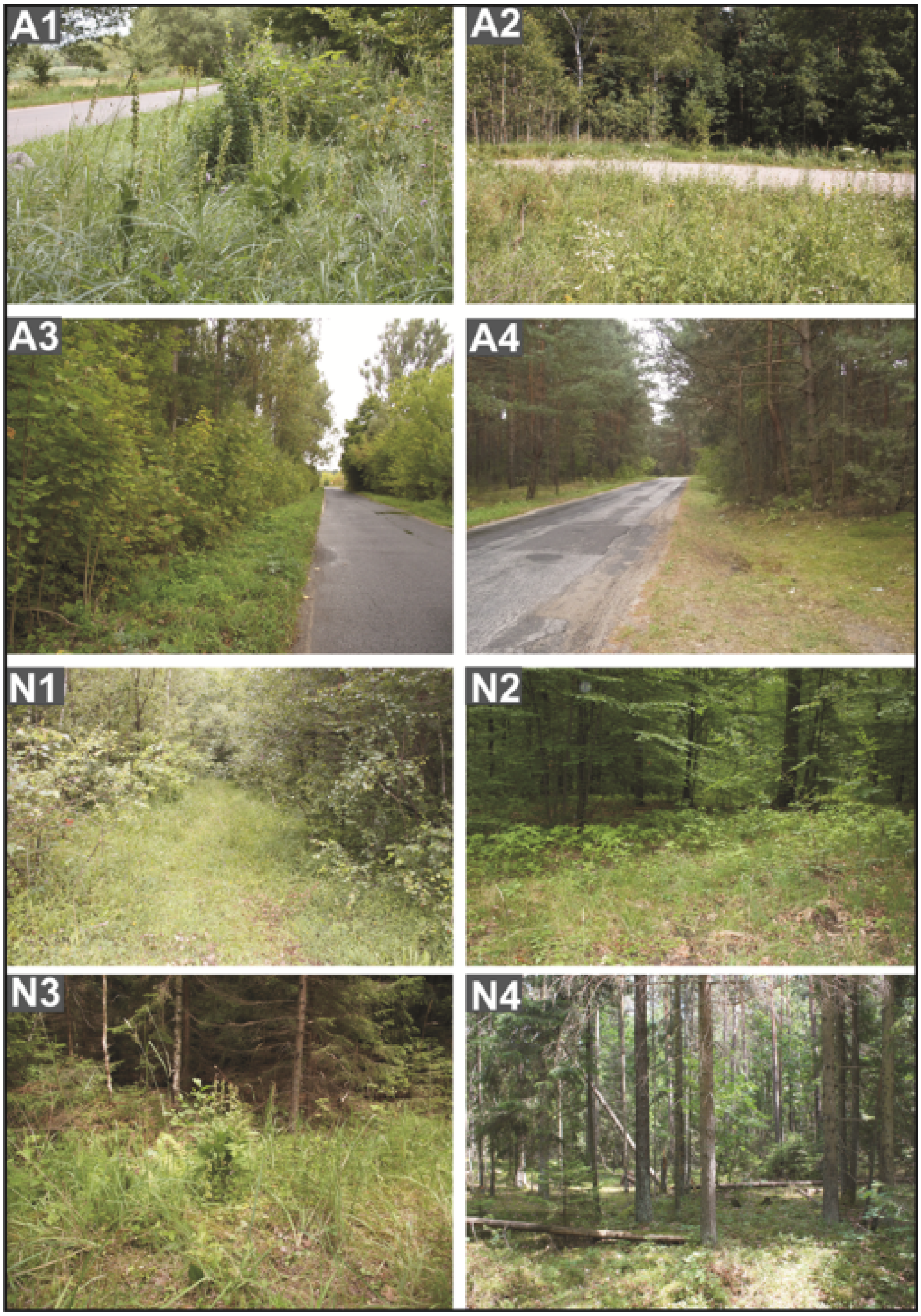


**Fig 1 Habitats of *E. helleborine*:** A – anthropogenic habitat, N – natural habitat, A1 – between the road and a wooden fence in a Guszczewina village, A2 – close to the car parking in Hajnówka, A3 – in the thicket by the roadside in Sulejów, A4 – on a roadside bordering pine forest in Sulejów, N1 – forest *Galio sylvatici-Carpinetum betuli* Oberd. 1957 in Kotowice, N2 –

forest *Galio-Carpinetum* Oberd. 1957 in Kaczawskie Mts., N3 and N4 – *Galio-Carpinetum* Oberd. 1957 in the Strict Reserve Bialowieża Primaeval Forest.

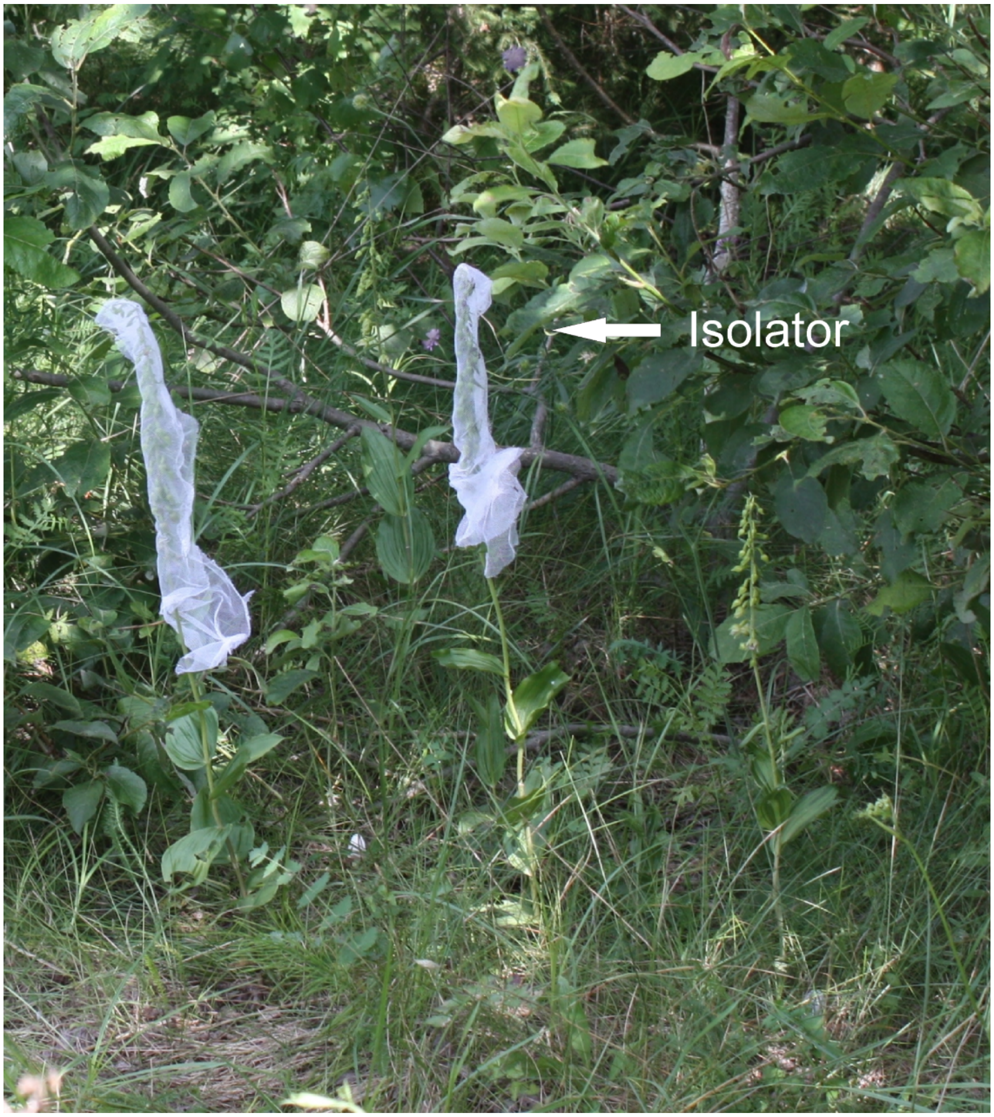


**Fig. 2 Isolators on shoots of *E. helleborine.***

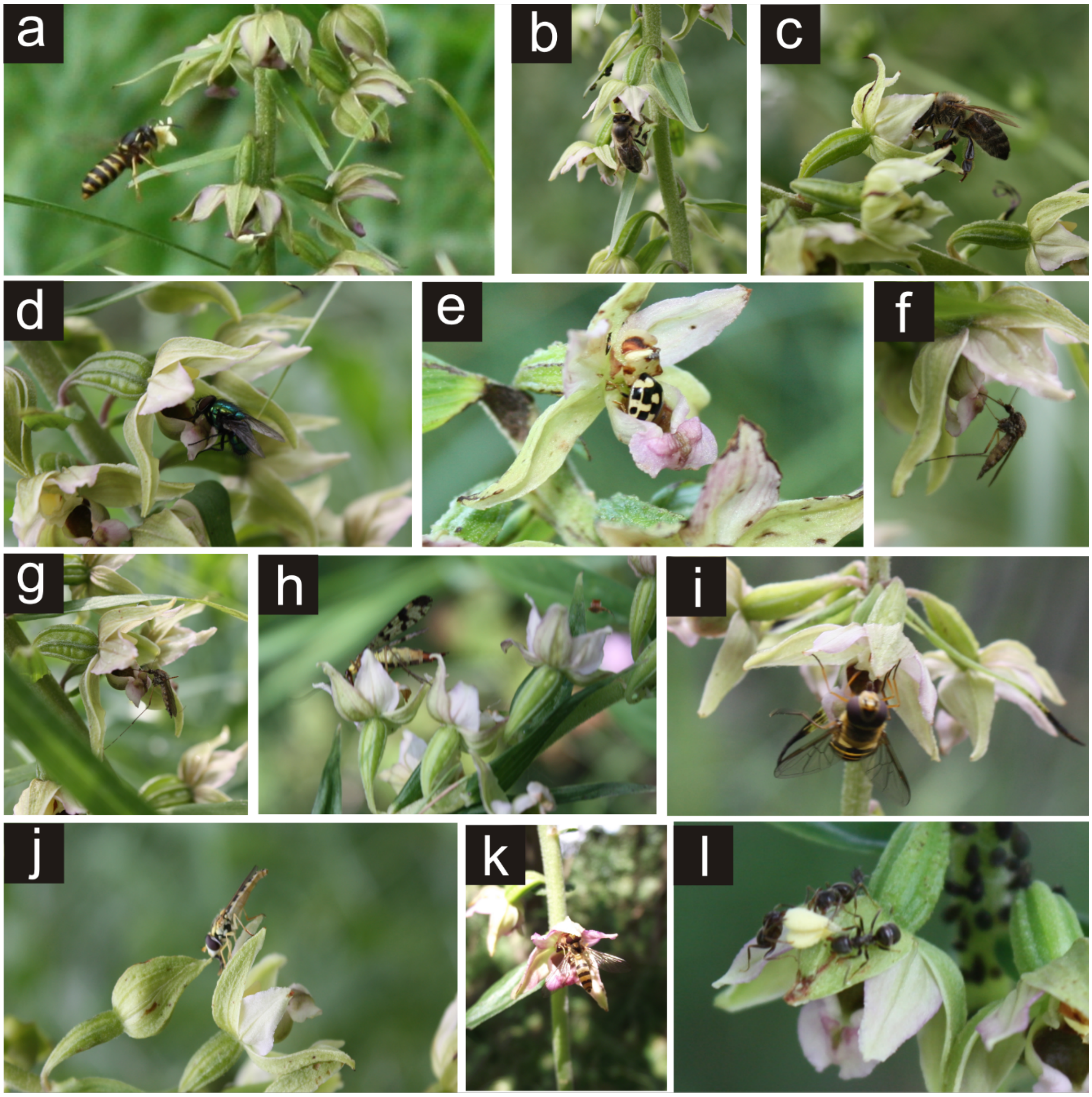


**Fig. 3 Pollinators of *E. helleborine***: a - wasp (Vespidae) with pollinia attached to head, b-c - honeybees (Apidae), d - carrion fly (Calliphoridae), e - ladybird (Coccinellidae), f-g - mosquito (Culicidae), h - scorpionfly (Panorpidae), i-k - hoverflies (Syrphidae), l - ants (Formicidae) (phot. A. Rewicz 2012/2013)

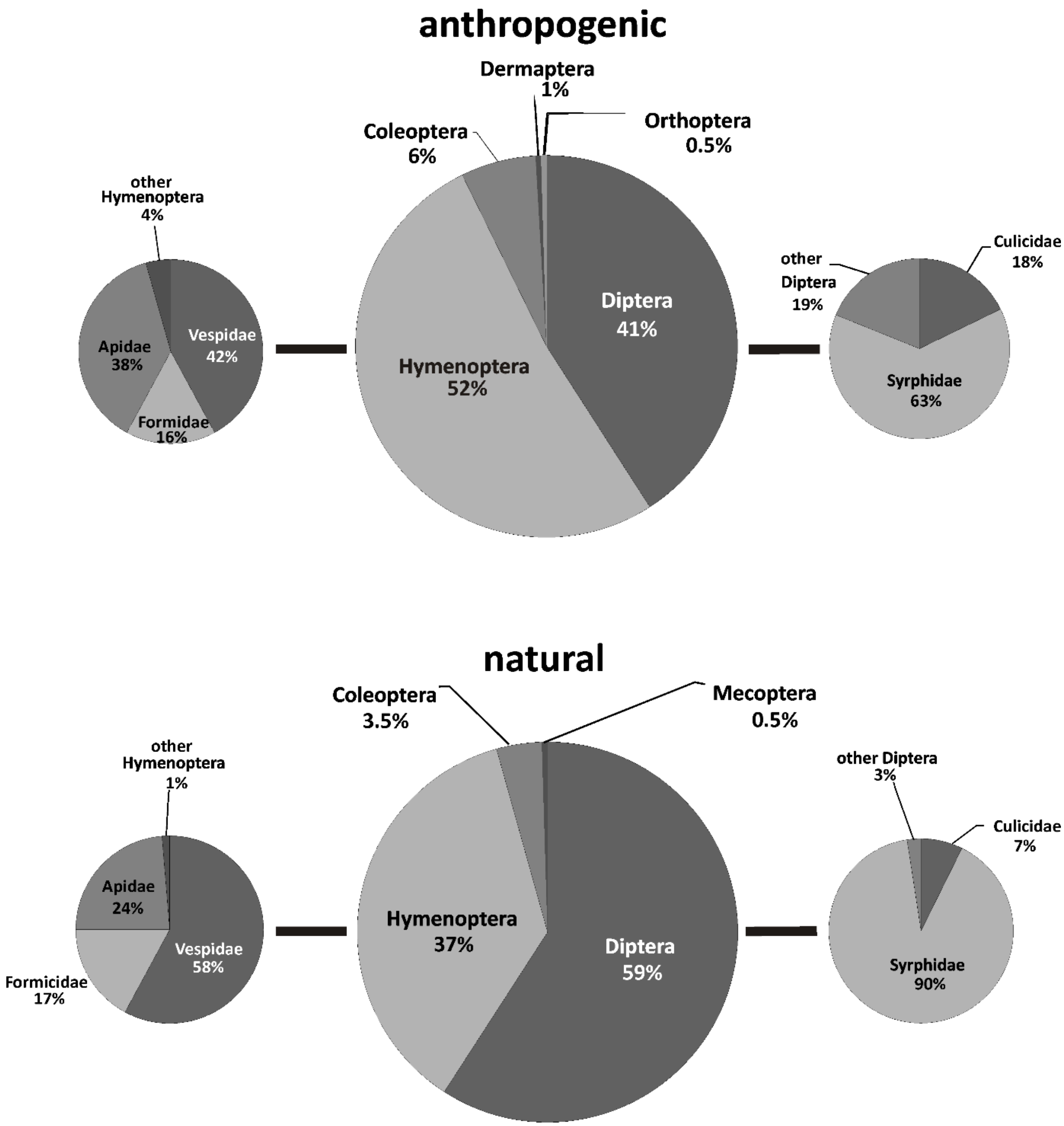


**Fig. 4 Taxonomic diversity of *E. helleborine* pollinators in natural and anthropogenic habitats.**

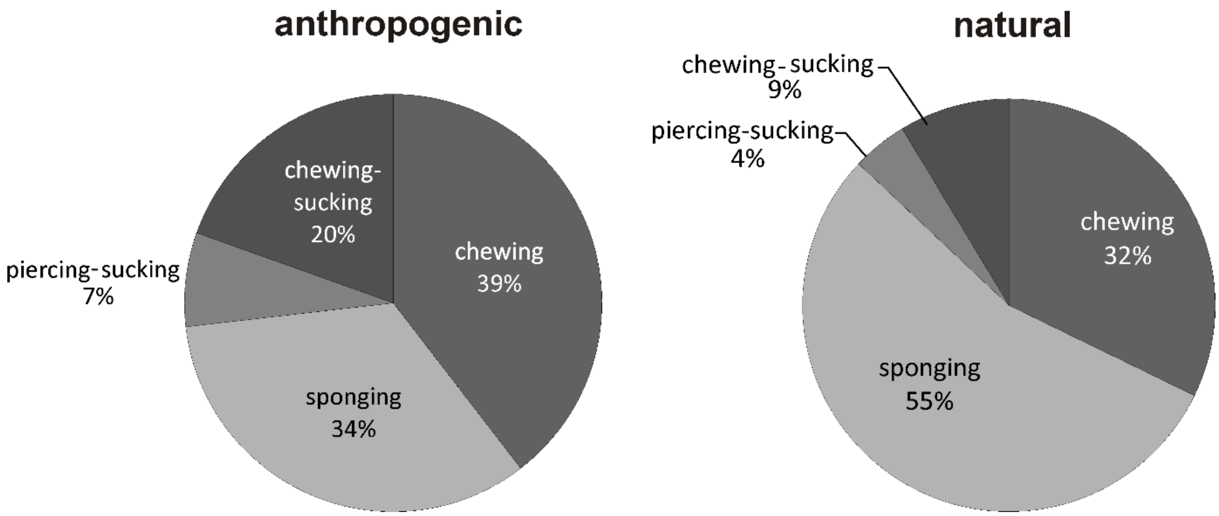


**Fig. 5 Diversity of *E. helleborine* pollinators in natural and anthropogenic habitats based on insect mouthparts**.

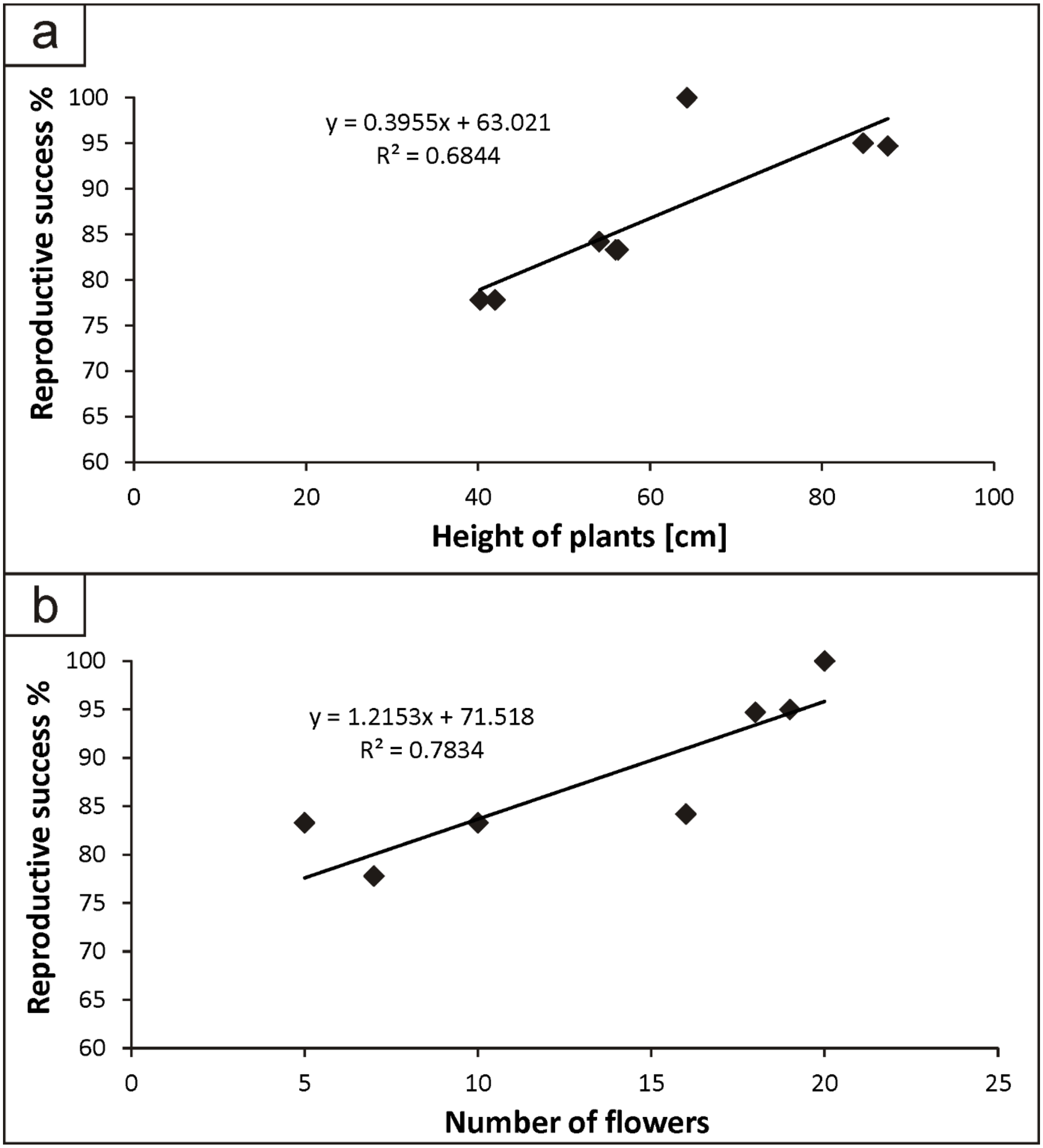


**Fig. 6 The dependence of reproductive success in anthropogenic populations:** a) the height of plants, b) number of flowers.

**Tab. 1 Studied populations of *Epipactis helleborine*.**

| Population code | locality | Population size ($m^2$) | Number of shoots | Density (shoots/$m^2$) | Coordinates |
|---|---|---|---|---|---|
| **anthropogenic habitats** | | | | | |
| A1* | roadside (Guszczewina) | 36 | 127 | 3.52 | N 52.831600<br>E 23.794836 |
| A2* | roadside (Hajnówka) | 108 | 102 | 0.94 | N 52.734217<br>E 23.603314 |
| A3 | roadside (Sulejów) | 460 | 80 | 0.17 | N 51.353793<br>E 19.883155 |
| A4 | roadside (Sulejów) | 46 | 152 | 3.30 | N 51.349757<br>E 19.882484 |
| **natural habitats** | | | | | |
| N1 | mixed forest (Kotowice) | 100 | 300 | 3.00 | N 50.963255<br>E 15.963255 |
| N2 | mixed forest (Kaczawskie Mts) | 40 | 150 | 3.75 | N 51.041241<br>E 17.176701 |
| N3* | mixed forest (Białowieża Primeval Forest) | 120 | 34 | 0.28 | N 52.828706<br>E 23.797095 |
| N4 | mixed forest (Białowieża Primeval Forest) | 400 | 41 | 0.10 | N 52.832427<br>E 23.763069 |

**Tab. 2 Pollinators of *Epipactis helleborine:*** C - chewing, S - sponging, PS - piercing and sucking, CS - chewing and sucking.

| Taxon of pollinator | | Type of mouthparts | Site type | |
|---|---|---|---|---|
| **Order** | **Family** | | **Natural** | **Anthropogenic** |
| Orthoptera | Acrididae | C | 0 | 1 |
| Dermaptera | Forficulidae | C | 0 | 1 |
| Diptera | Calliphoridae | S | 1 | 7 |
| | Culicidae | PS | 9 | 16 |
| | Lauxanidae | S | 0 | 1 |
| | Muscidae | S | 1 | 3 |
| | Scathopagidae | S | 0 | 1 |
| | Sepsidae | S | 0 | 1 |
| | Syrphidae | S | 111 | 57 |
| | Tachinidae | S | 1 | 2 |
| | Tephritidae | S | 0 | 1 |
| | Tipulidae | S | 0 | 1 |
| Mecoptera | Planorpidae | C | 4 | 0 |
| Hymenoptera | Apidae | CS | 18 | 43 |
| | Formicidae | C | 13 | 18 |
| | Ichneumonidae | C | 1 | 4 |
| | Pamphiliidae | C | 0 | 1 |
| | Vespidae | C | 44 | 48 |
| Coleoptera | Cantharidae | C | 0 | 10 |
| | Cerambycidae | C | 1 | 0 |
| | Coccinellidae | C | 0 | 4 |
| | Elateridae | C | 2 | 0 |
| | Melyridae | C | 4 | 0 |
| | Nitidulidae | C | 1 | 0 |
| | Total | | 208 | 220 |

**Tab. 3 Mean number of flowers and fruits in analyzed populations and Spearman correlation between number of flowers and fruits.**

| Populations | Number of flowers | Number of fruits | Correlation |
|---|---|---|---|
| Natural pollination (allogamy) | | | |
| Anthropogenic | 22 | 19 | r=0.96 |
| Natural | 21 | 19 | r=0.98 |
| Autogamy | | | |
| Anthropogenic | 24 | 20 | r=0.88 |
| Natural | 15 | 12 | r=0.88 |

**Tab. 4. Ratio of dead and alive seeds developed in autogamy and natural pollination in analyzed populations** (ns – Non-significant results).

| Trait | Populations | | U Mann-Whitney test, p<0.05 |
|---|---|---|---|
| | Anthropogenic | Natural | |
| | Natural pollination (allogamy) | | |
| live seed (%) | 49.7 | 51.2 | Ns |
| dead seeds (%) | 50.3 | 48.8 | Ns |
| | Autogamy | | |
| live seed (%) | 24.6 | 29.5 | p<0.05 |
| dead seeds (%) | 75.4 | 70.5 | p<0.05 |

**Tab. 5 Reproductive success of *Epipactis helleborine* in natural and anthropogenic populations:** D(n/$m^2$) - density of population, HP - height of plants, NFS - number of flowering shoots, NF - number of flowers, NFR - number of fruits, SR - reproductive success, ± standard deviation

| Anthropogenic habitats | | | | | | | Natural habitats | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2011 | | | | | | | 2011 | | | | | | |
| site | D (n/$m^2$) | HP | NFS | NF | NFR | RS | site | D (n/$m^2$) | HP | NFS | NF | NFR | RS |
| A1 | 0.28 | 84.79 | 39 | 20±9.20 | 19±9.48 | 95.00 | N1 | 0.83 | 61.96 | 25 | 18±11.8 | 8±7.80 | 44.40 |
| A2 | 0.93 | 54.08 | 26 | 19±8.63 | 16±7.83 | 84.20 | N2 | 0.77 | 57.36 | 30 | 18±10.8 | 13±8.36 | 72.20 |
| A3 | 0.9 | 56.29 | 22 | 12±6.20 | 10±5.92 | 83.30 | N3 | 0.21 | 34.53 | 28 | 18±12.5 | 14±11.99 | 77.80 |
| A4 | 0.4 | 41.98 | 38 | 9±9.25 | 7±9.21 | 77.80 | N4 | 0.88 | 48.36 | 33 | 24±14.8 | 20±13.15 | 83.30 |
| Average | 0.63 | 59.28 | 31.25 | 15 | 13 | 85.08 | Average | 0.67 | 50.55 | 29 | 20 | 14 | 69.42 |
| 2012 | | | | | | | 2012 | | | | | | |
| A1 | 0.38 | 87.65 | 43 | 19±8.37 | 18±8.28 | 94.70 | N1 | 1.24 | 66.67 | 15 | 20±6.34 | 17±5.19 | 85.00 |
| A2 | 0.77 | 64.3 | 45 | 20±14.1 | 20±13.87 | 100.00 | N2 | 0.97 | 62.15 | 24 | 19±11.4 | 14±10.00 | 73.70 |
| A3 | 2.39 | 56 | 13 | 6±6.20 | 5±5.81 | 83.30 | N3 | 0.22 | 55.22 | 25 | 24±13.4 | 15±10.45 | 62.50 |
| A4 | 0.33 | 40.24 | 46 | 9±8.73 | 7±8.66 | 77.80 | N4 | 0.81 | 46.07 | 32 | 25±14.3 | 20±14.00 | 80.00 |
| Average | 0.97 | 62.05 | 36,75 | 14 | 12 | 88.95 | Average | 0.81 | 57.53 | 24 | 22 | 17 | 75.30 |