

Is this a new species, hybrid or maybe phenotypic plasticity results? The role and significance of phenotypic plasticity of flowers in taxonomy of *Dactylorhiza majalis* (Rchb.) P. F. Hunt & Summerh. (Orchidaceae)

Nowy gatunek, mieszaniec czy efekt plastyczności fenotypowej? Rola i znaczenie plastyczności fenotypowej kwiatów w taksonomii *Dactylorhiza majalis* (Rchb.) P. F. Hunt & Summerh. (Orchidaceae)

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ABSTRACT: In the paper we present the results of studies on the variability range of the flower colour, size and shape in *D. majalis*. The research was carried out in three populations, deliberately chosen so that to exclude the possibility of hybridisation with other orchid species. The obtained results confirm a wide range of phenotypic plasticity of a taxon, in extreme cases causing problems in species identification. The performed analyses demonstrate that the labellum index, similarly to the colour of the flowers and the shape and the pattern of labellum, have a wide range of individual variability, which makes it quite difficult to classify taxa within the genus.

KEY WORDS: *Dactylorhiza majalis*, phenotypic plasticity, diversity

Introduction

Phenotypic plasticity has become a quite trendy issue in scientific research recently (e.g. Schlichting 2002). The term has been known to scientists since

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last century and the first who attempted to define this phenomenon was Bradshaw (1965). As phenotypic plasticity he recognized the phenotypic result of genotypic properties, i.e. the ability of a genotype to modify its phenotype under environmental conditions. Phenotypic variability derives from the fact that the same set of genes can produce different phenotypes under diverse environmental conditions (Wojciechowska 2002).

Thanks to the development of investigative techniques we know that it is genetics that underlies the phenomenon. Three groups of genes are responsible for morphologic plasticity: allelic sensitivity, plasticity and transduction genes (Pigliucci, Byrd 1998). Molecular basis of this phenomenon can also be determined by varied gene regulation belonging to multigene families, particularly MADS-box homeotic genes (Smith 1990).

Great individual variability in *Dactylorhiza*, incidentally causing diagnostic problems, results from different sources (Heslop-Harrison 1968). Among factors which undoubtedly influence plant morphology are for instance chemical composition and insolation of a habitat. Genetic factors such as gene expression, mutations or recombination are also of considerable significance here.

In taxonomic researches, and especially in species identification process, a lot of attention is given to morphologic features: particularly to the colour and size of flowers as well as the labellum index and the shape of leaves, which in case of *Dactylorhiza* Necker ex Nevski genus occasionally becomes troublesome.

The objective of this paper is to visualize the range of phenotypic variability and an attempt to establish frames of this phenomenon for Western Marsh-orchid.

1. Materials and methods

The research was conducted in the years 2007–2008 in chosen populations in Poland, i.e. in the Orlickie Foothills (Duszniki Zdrój, ATPOL **BF 24**), the Sowie Mountains (Walim, ATPOL **BE 94**) and Suwałki Landscape Park (ATPOL **FA 98**).

The populations were purposely chosen for their geographical location as well as specific climatic and habitat conditions.

In order to compare the colouration of individual flowers, and particularly of the labella, photographs were taken in the field.

Hand-made drawings of the photographic documentation of the flowers were also made to be able to compare their shapes. The labellum index was calculated for the examined specimens in populations, according to the generally accepted Heslop-Harrison formula (Pedersen 2004).

The research activities were conducted noninvasively as the studied taxon is protected in Poland.

2. Results

The colour of flowers

On the basis of the conducted research it has been found that flowers of *Dactylorhiza majalis* are in different shades of red: starting with light pink through magenta to violet (Plate 1, Fig. A, B, C, D, E, F, G, H, I, J). There were also rare hypochromic specimens (Plate 1, Fig. L).

The commonest in populations were specimens with average features.

Not a high range of variability was found in case of other petals of perianths. They were usually of the same colour as the labellum (Plate 1, Fig. B, F). In specimens with white labella, the remaining petals were pink (Plate 1, Fig. K).

The type of pattern on the labellum

As a result of performed analyses it has been found that the majority of flowers possess a distinct pattern (red markings) on the labellum, whose colour was noticeably darker than the remaining elements of a flower (Plate 1, Fig. B, C, D).

Three types of pattern were found: linear, punctual, mixed and a very rare variant – lacking in pattern (Fig. 1, Plate 1). The mixed pattern on labella was predominant in all the populations.

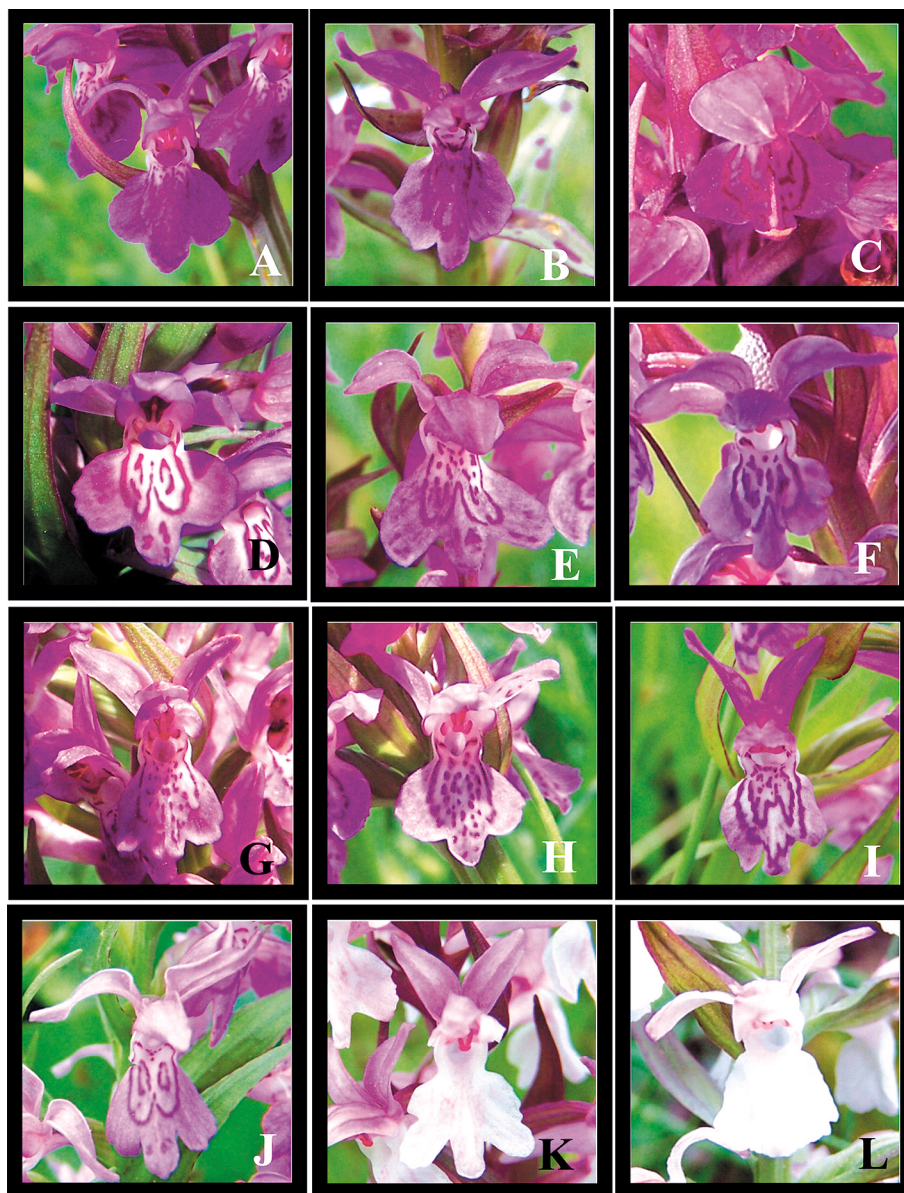
The shape of flowers

While analysing the flower shape, a particular attention was given to the shape of the labellum. As a result of our observations it has been noticed that the labella are usually rhomboidal, trilobed with clearly marked median and lateral lobes.

A small number of flowers in Suwałki Landscape Park were flowers whose labellum was not very wide and without a clear division into lobes (Plate 2, Fig. K).

In Walim population an atypical flower was observed, with a triangular and not divided into lobes labellum, formed probably as a result of ontogenetic distortions (Plate 2, Fig. L).

In the course of the research it has also been discovered that in some cases the median lobe was considerably elongated (Plate 2, Fig. B, H) and could take different shapes although specimens with a rounded median lobe (Plate 2,



— 0,5 cm

Plate 1. Individual phenotypic variability of *Dactylorhiza majalis* (Rchb.) P. F. Hunt & Summerh. of flower colours and type of marked labellum (Phot. A. Faltyn)

Tablica 1. Zmienność fenotypowa barwy kwiatów i typu wzoru warżki osobników *Dactylorhiza majalis* (Rchb.) P. F. Hunt & Summerh. (Fot. A. Faltyn)

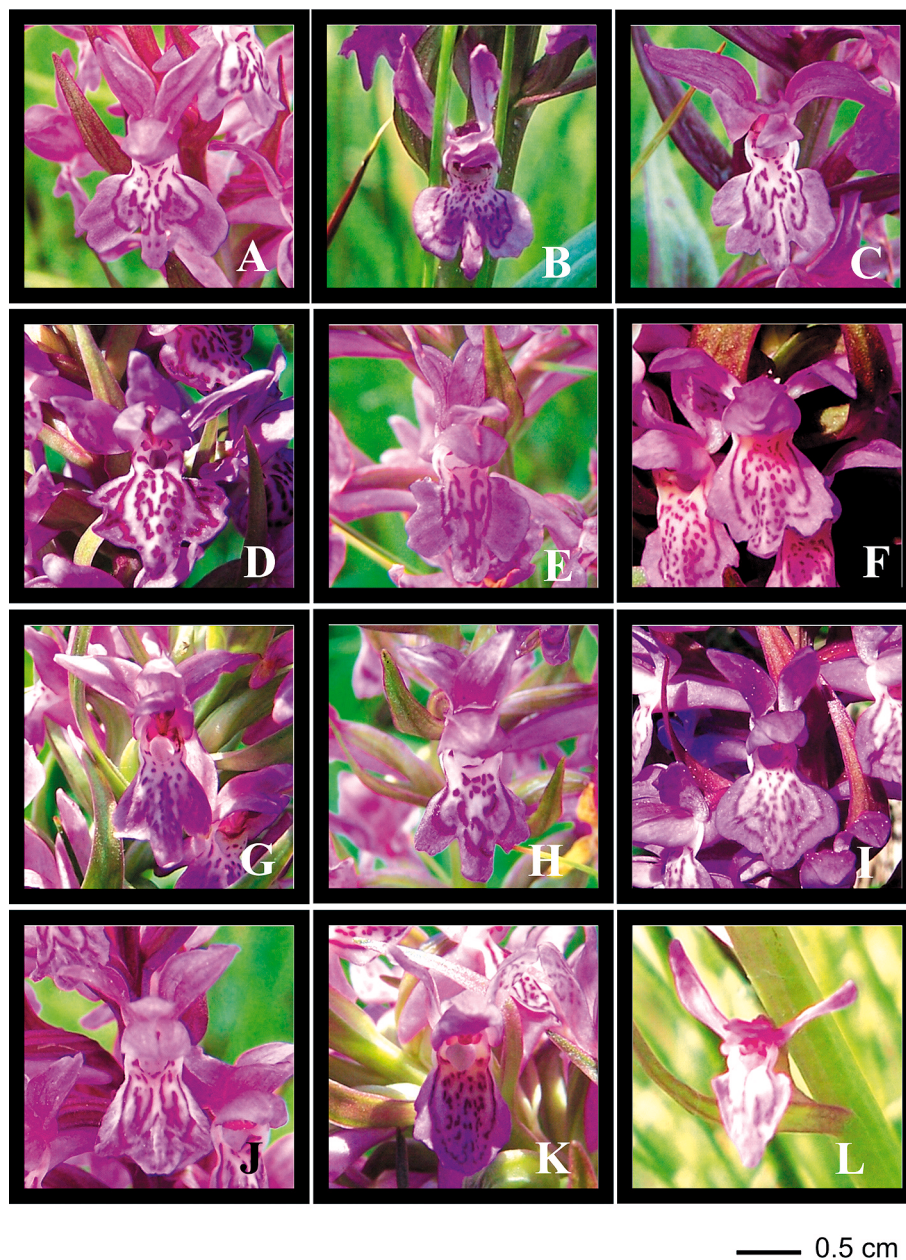


Plate 2. Individual phenotypic variability of *Dactylorhiza majalis* (Rchb.) P. F. Hunt & Summerh. of labellum shape (Phot. A. Faltyn)

Tablica 2. Zmienność fenotypowa kształtu warzki osobników *Dactylorhiza majalis* (Rchb.) P. F. Hunt & Summerh. (Fot. A. Faltyn)

Fig. A) were predominant in the populations while those with triangular median lobes were rarely observed.

The edges of lateral lobes were indented or entire (Plate 2).

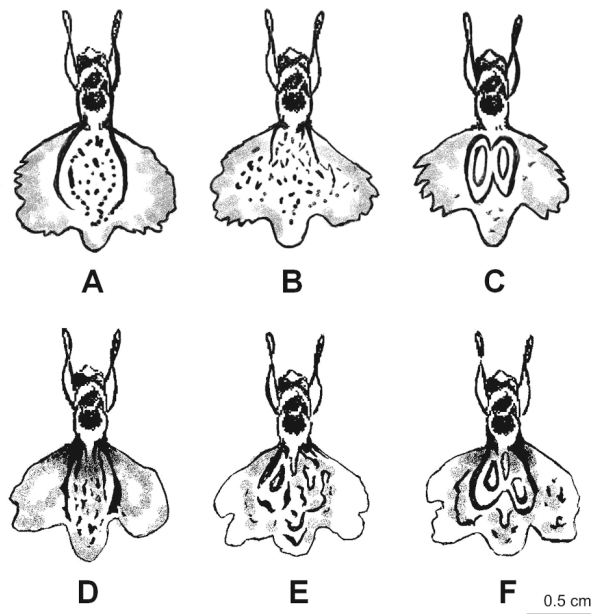


Fig. 1. The types of patterns found on labellum in the course of the research
Ryc.1. Różne typy wzoru na warzce stwierdzone w trakcie badań

The labellum index

In order to more exactly compare the similarities and differences in the morphology of flowers in particular populations, the labellum index was calculated for every flower according to the Heslop-Harrison formula (Pedersen 2004)

The majority of the labellum index results had average values for the given ranges.

The range of variability was observed in a very numerous population of *Dactylorhiza majalis* in the Sowie Mountains and amounted 0.56–0.87. Equally wide variability was observed in a considerably less numerous *Dactylorhiza majalis* population in Duszniki Zdrój and a numerous population in Suwałki Landscape Park. In those populations the range of variability was respectively 0.62–0.91 and 0.61–0.92.

Tab. 1. The results of the labellum index in the individual populations, given in ranges from the minimum to the maximum value

Tab. 1. Wyniki obliczeń indeksu warżki w poszczególnych populacjach podane w zakresach od wartości minimalnej do wartości maksymalnej

Site	Minimum	Maximum
Suwałki Landscape Park (ATPOL FA 98)	0.61	0.92
The Sowie Mts. (Walim) (ATPOL BE 94)	0.56	0.87
The Orlickie Foothills, Duszniki Zdrój (ATPOL BF 24)	0.62	0.91

3. Discussion

Individual populations vary considerably in respect of the colour of the labellum and the pattern on it. No two identical specimens in respect of the colour and shape of the labellum as well as its pattern were found in the populations although the colour of the flowers and the pattern on the labellum are used as one of the diagnostic features. Additional difficulty in marking individual specimens is the possibility of occurrence of flowers different in size and colouration within an inflorescence, which is probably related to the developmental biology of specimens.

When many specimens of different phenotype grow in one place, it frequently becomes problematic whether the morphologic differences are merely a result of variability or whether the plants belong to separate taxonomic units. An example of such situation is the population in Walim in the Sowie Mountains where one of the specimens had unique flowers with no clear division of the labellum (Plate 2, Fig. L). It is possible, however, that in case of that specimen and of another one from this population it is merely a result of morphogenetic development anomalies. It definitely constitutes an impediment in taxonomic researches.

Some of the examined phenotypes of individual specimens resemble *D. traunsteineri*, *D. pratermissa* or *D. cordigera* (e.g. Plate 2, Fig. I, K), which is, unfortunately, absolutely impossible as these taxa do not occur in the said area. Such similarities should be regarded as accidental, and attributed most probably to the joint influence of genetic, habitat and climatic factors, with no taxonomic significance. Specimens of this type were very rarely found, usually in particularly numerous populations of *D. majalis* and accounted for ca. 0.01%.

Basing on the shape of the labellum some flowers in the examined populations could have been identified as separate species or subspecies. It should therefore be considered whether isolating individual units solely on the ground of analysis of (very changeable) morphologic features is reasonable.

The labellum index used by some researches as a diagnostic feature has also proved deceptive as it, similarly to the flower colour, has a wide range of values within the genus and does not allow to give a definite diagnosis.

It should be seriously considered whether to isolate subspecies as well as forms and varieties of individual *Dactylorhiza* groups, which is consistent with the data obtained by Tukałło and Minasiewicz (2003).

In case of the *Dactylorhiza* genus it is not only difficult to mark individuals but to establish definitions of species, subspecies and varieties, which would help organize its taxonomy (Pedersen 2004). Determining the rank of individual taxa, by some thought to be subspecies and by others treated as separate species, also poses a problem.

In his work (1998) Pedersen gives definitions of separate taxonomic units for Western marsh-orchid. According to this author species are “designated taxa complying with the biological species concept in a modern, botanically focused sense”, subspecies are “designated taxa complying with the ecological, but not with the biological species concept” while varieties are “designated taxa complying with the phenetic, but neither with the biological nor the ecological species concept.”

Acknowledging the significance of the variability phenomenon in taxonomy of phenotypically plastic genera such as *Dactylorhiza* is crucial for Orchidaceae taxonomy.

Prior to establishing diagnoses or, what is more essential, prior to designating a new taxon, we should consider whether the observed effect of “diversity” of a specimen is not the result of distortions in morphogenetic development or is not caused by the synergetic effect of genetic factors and edafic conditions.

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References

- BRADSHAW A. D. 1965. Evolutionary significance of phenotypic plasticity in plants. – *Adv. Gen.* **13**: 115–155.
- HESLOP-HARRISON J. 1968. Genetic systems and ecological habit as factors in dactylorchid variation. – *Jahresberichte der Naturwissenschaftlichen Vereins in Wuppertal* **21/22**: 19–27.
- PEDERSEN H. Æ. 1998. Species concept and guidelines for infraspecific taxonomic ranking in *Dactylorhiza* (Orchidaceae). – *Nordic J. Bot.* **18**: 289–310.
- PEDERSEN H. Æ. 2004. *Dactylorhiza majalis* s.l. (Orchidaceae) in acid habitats: variation patterns, taxonomy, and evolution. – *Nordic J. Bot.* **22**: 641–658.
- PIGLIUCCI M., BYRD N. 1998. Genetics and evolution of phenotypic plasticity to nutrient stress in *Arabidopsis*: drift, constraints or selection? – *Biol. J. Linn. Soc.* **64**: 17–40.
- SCHLICHTING C. D. 2002. Phenotypic plasticity in plants. – *Plant Species Biology* **17**: 85–88.
- SMITH H. 1990. Signal perception, different expression within multigene families and the molecular basis of phenotypic plasticity. – *Plant Cell Envir.* **13**: 585–594.
- TUKAŁŁO P., MINASIEWICZ J. 2003. Zmienność morfologiczna a problemy klasyfikacji taksonów krytycznych w rodzaju *Dactylorhiza* (Orchidaceae). – I Kongres Taksonomii Polskiej. Genus, Supplement, Wrocław, s. 77–80.
- WOJCIECHOWSKA A. 2002. Plastyczność morfologiczna – podstawy genetyczne i ewolucja. – *Wiadomości Botaniczne* **46**(1/2): 27–34.

Streszczenie

Przedmiotem pracy było określenie roli i znaczenia zjawiska plastyczności fenotypowej w taksonomii *Dactylorhiza majalis* (Rchb.) P. F. Hunt & Summerh. Necker. Nieinwazyjne badania, polegające na dokumentowaniu wyglądu, barwy, kształtu oraz rysunku na warżce prowadzono w latach 2007–2008, w trzech populacjach tj. na Pogórzu Orlickim, na terenie Suwalskiego Parku Krajobrazowego oraz w Górach Sowich. Populacje te wybrano celowo ze względu na ich dużą liczebność, położenie geograficzne, warunki klimatyczne a także brak możliwości hybrydyzacji z innymi taksonami, co mogłoby wpłynąć na obserwowany fenotyp.

Stwierdzono duży zakres zmienności fenotypowej, zarówno pod względem kształtu jak i barwy okwiatu. Kwiaty posiadały odcień od purpurowoczerwonego aż po fioletowy. Jedynie w jednej populacji znaleziono kwiat biały, nieposiadający barwnego wzoru na warżce – efekt hypochromii.

W populacjach stwierdzono również kwiaty nieposiadające typowego dla tego gatunku kształtu warżki, co należy uznać najprawdopodobniej za wynik zaburzeń morfogenetycznych, lub zaburzeń w działaniu genów odpowiedzialnych za wykształcenie tych elementów warżki.

Na podstawie przeprowadzonych badań zauważono, iż we wszystkich populacjach pojawiły się trzy typy wzorów na warżce, tj.: liniowy, punktowy, oraz mieszany – będący połączeniem dwóch poprzednich. Niewielki procent stanowiły kwiaty całkowicie pozbawione wzoru na warżce.

Przeprowadzone badania wykazały duży zakres plastyczności fenotypowej taksonu utrudniający klasyfikację oraz często powodujący błędne oznaczenie materiału. Uzyskane wyniki potwierdzają problematyczny status taksonu, oraz trudność w ustaleniu cech istotnych taksonomicznie, a więc takich, które cechowałby najmniejszy zakres zmienności.