

**INTRODUCTION**

*Dactylorhiza* Nevski is distributed across temperate and subtropical Eurasia and North Africa. The number of accepted species varies from 12 to 75 (Pedersen, 1998). There are around 12 *Dactylorhiza* species in European Russia and most of them have probably a West European origin. Investigations of allozyme markers and AFLP (Hedren, 2002) showed that part of *Dactylorhiza* is **diploid** (e.g., *D. incarnata*), another part is **autopolyploid** (*D. maculata*) and third has **allotetraploid** origin (*D. traunsteineri* and others).

Microsatellites are short repeats in DNA that have high diversity on species level and thus can be useful for classification of closely related species and determining origin and geographical patterns. Orchid plastid microsatellites can also help to determine possible maternal parents of allopolyploids. Microsatellite study of Russian *Dactylorhiza* was never performed. The goal of our work is to screen plastid microsatellites and then to compare results with West European data.

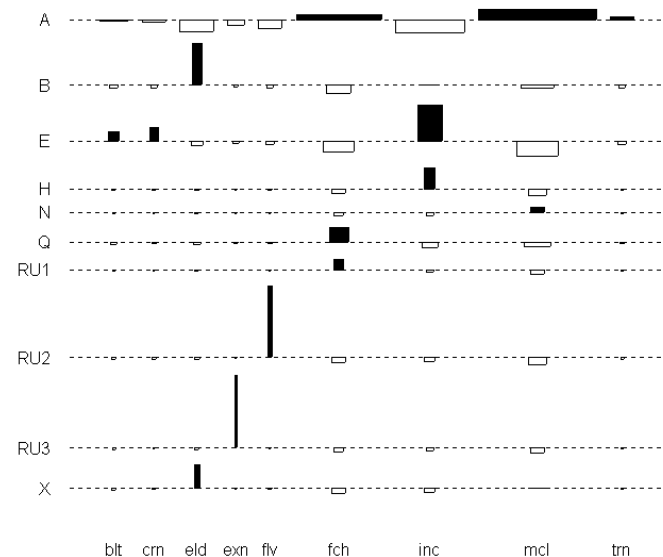
**MATERIAL AND METHODS**

107 samples were collected in different parts of European Russia and West Caucasus, from Krasnodar to Murmansk region—more than 3000 km from south to north. Then samples were dried in silica gel to prevent DNA degradation. DNA was extracted by common CTAB protocol (Doyle and Doyle, 1987). PCR was performed with set of primers to amplify four polymorphic loci in three different plastid DNA regions: *trnS-trnG* spacer, *trnL* intron and *trnL-trnF* spacer.

**RESULTS AND DISCUSSION**

We found ten combinations of fragment lengths, referred below as “haplotypes”. In general, our data strongly coincide with analogous West European research. Among these haplotypes *D. fuschii* (A) haplotype is most frequent.

We found also three specific haplotypes: RU1, RU2 and RU3. The first probably has independent origin from the typical for this region (North Karelia) *D. fuschii* haplotype. RU2 is from specimens determined as *D. flavescens* and close to *D. romana* haplotypes from Mediterranean region. RU3 haplotype from *D. euxina* is close (but not identical) to two haplotypes reported from samples of this species in Turkey.



**Fig. 1:** Association between haplotypes and species. Length and width of black squares indicate positive  $\chi^2$  value and sample numbers, respectively. Species names are abbreviated to three letters.

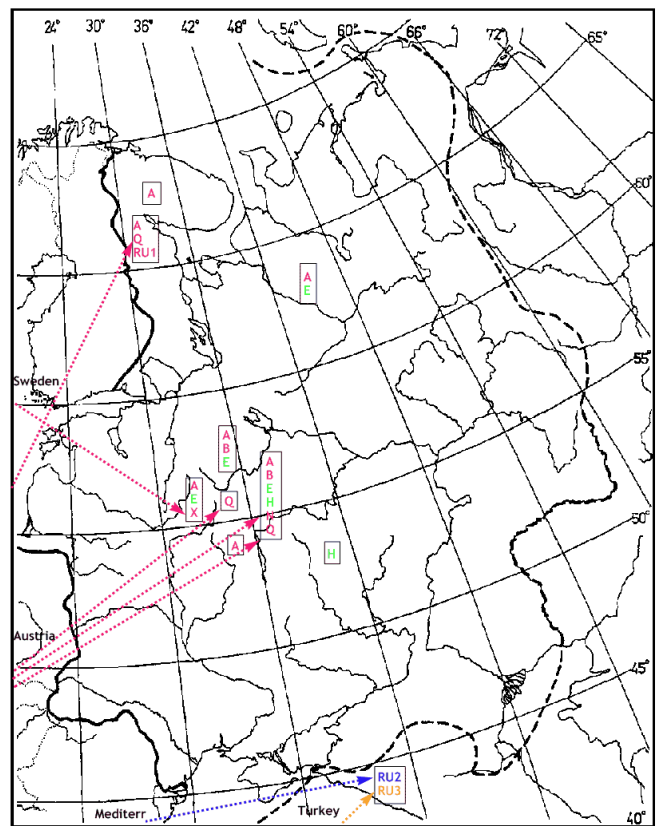
There is strong association between species and haplotype, with few exceptions (fig. 1). Surprisingly, most North Russian *D. maculata* specimens share their haplotype with *D. fuschii*, not with typical for *D. maculata* s.str. B haplotype. However, most of this plants growth in the *Sphagnum*-bogs, which are typical for *D. maculata*. This situation might have several explanations: (1) misidentification, (2) introgression from *D. fuschii* at the border of area or (3) independent origin of *D. maculata* in Northern Europe. *Dactylorhiza elodes* samples have association with typical *D. maculata* (B) haplotype,

thus Russian *D. elodes* can belong to *D. maculata*.

*Dactylorhiza cruenta* now often accepted as form of *D. incarnata* (see, for example, Hedren et al., 2001), which coincides with our data because all our *D. cruenta* samples contain typical for *D. incarnata* haplotype E. Haplotype H (initially founded in Georgian material) was found in typical *D. incarnata* samples from Central Russia. This haplotype (and also Q and X) probably have wider distribution than was previously imagined.

Interestingly also is the absence of haplotype C, reported for European *D. traunsteineri* and other allotetraploids. All our possible tetraploid specimens shared haplotypes either with *D. fuschii* A (for *D. traunsteineri*) or with *D. incarnata* E (for *D. baltica*). In addition, haplotypes N and X from Western Europe often belong to allotetraploids and thus another undescribed allotetraploid form could exist in Russia.

We founded significant geographical pattern of haplotype distribution from south to north (Kruskal-Wallis  $\chi^2 = 21.4529$ , p-value  $\ll 0.05$ ). Distribution of haplotypes in Russia also has links to Western Europe flora (fig. 2). These observations support Averjanov (1990) point of view for main areas of *Dactylorhiza* distribution.



**Fig 2:** Map of European Russia with distribution of founded haplotypes (collection points are in rectangle centres).

**CONCLUSIONS**

1. The main features of haplotypes existence and distribution in European Russia coincide with Western European data.
2. Most *D. maculata* specimens from Russia have *D. fuschii* haplotype. This can be evidence for introgression or independent origin of *D. maculata*-like forms.
3. Several haplotypes uncommon for the West are more widely distributed in Russia whereas some frequent haplotypes doesn't exist.
4. There is clear difference between haplotype frequencies alongside south-north axis.

**SELECTED REFERENCES**

Averjanov L. (1990). *Novosti sistematiki vysshikh rastenij*. 27: 32–62 (in Russian).  
 Hedrén M., M. F. Fay and M. W. Chase (2001). *Amer. J. Bot.* 88(10): 1868–1880.  
 Hedrén M. (2002). *Jour. Eur. Orch.* 34(4): 707–731.  
 Pedersen H. A. (1998) *Nord. J. Bot.* 18(3): 289–310.