

Floral polymorphism in common primrose (*Primula vulgaris* Huds., Primulaceae) of the North-Eastern Black Sea coast

Alexey Shipunov, Yana Kosenko, Polina Volkova

The common primrose (*Primula vulgaris*, Primulaceae) has several color morphs. Two of them, traditionally treated as subspecies, *vulgaris* with white and yellow flowers, and polymorphic *sibthorpii* with the predominance of dark (anthocyanin-containing) flowers, often occur on the same places along the eastern Black Sea coast, stretching over 250 km between Novorossiysk and Pitsunda. Seventy-one primrose populations were sampled on almost every 10-km interval of this line. We found a significant trend of increase in the proportion of dark flowers in the populations from north-west to south-east, with an abrupt (60 km) transitional zone. Within this zone, we found significant spatial trends correlated with altitude and distance from seashore. No reliable morphological differences between color forms were found. The observed large-scale geographical structure may be a joint result of the recent contact of previously isolated color morphs, heterogeneity of pollinator preferences, and genetic drift.

Key words: *Primula*; flower color polymorphism; Black Sea; Caucasus; geography; taxonomy

Suggested running title: Floral polymorphisms in *Primula vulgaris*

Primroses (*Primula* L., Primulaceae) are remarkable plants with features making them appropriate candidates for a wide range of scientific research, especially in population biology, evolution of sex, plant-animal interactions, and geographic diversity (Valentine, 1947, 1948 and 1955; Smith & Fletcher, 1948; Whale, 1984; Mast et al. 2001; Richards, 2003; Kalman et al., 2004; Guggisberg et al., 2006; Kalman et al., 2007). One of the common European primroses, *P. vulgaris* Huds. (= *P. acaulis* (L.) Hill.) has attracted the attention of botanists and geneticists for almost a century (Marsden-Jones & Turrill, 1944; Valentine, 1947, 1948 and 1955; Boyd et al., 1990; Karlsson, 2002). The main reason is that this species is heterostylous and therefore two different sexual forms, with long and short styles, occur in every population. Moreover, this particular species expresses not only style dimorphism, but also flower color polymorphism. *Primula vulgaris* is widespread in outer regions of Western Europe, the Mediterranean (including North Africa), southwestern Ukraine, the Crimea, Caucasus, and on the southern shore of the Caspian Sea. Throughout most of the European part of its range, the common primrose is more or less monochromic (usually with yellow flowers), whereas in eastern regions (Caucasus, Greece, Turkey, Iran, but not Crimea) plants are remarkably polymorphic: in addition to yellow-flowering plants, plants with white, pink, violet, and purple flowers of different tones are present in most of the populations (Smith & Fletcher, 1948; Fedorov, 1952; Zernov, 2002; Richards, 2003).

Northwestern Transcaucasia is an especially interesting study site of primrose diversity, because populations' flower colors change along the Black Sea coast. From Novorossiysk to Pitsunda (250 km distance), the color changes from predominantly yellow to predominantly purple (Richards, 2003). This difference has been long considered to be of taxonomical importance: various monographers accept plants with dark (anthocyanin-containing, see Table 2) flowers as a separate subspecies, *P. vulgaris* subsp. *sibthorpii* (Hoffm.) Smith & Forrest (Smith & Fletcher, 1948; Richards, 2003), or even as a separate species, *P. sibthorpii* Hoffm. (Fedorov, 1952 and 1973; Kosenko, 1970). It has also been noted that the latter form usually grows at lower altitudes (Kolakovskiy, 1985; Richards, 2003); this was also mentioned in other parts of *P. vulgaris* s.l. area, notably in Greece and Turkey (Richards, 2003). Some researchers divided Caucasian primroses into more species according the expression of different colors: *P. komarovii* Lozina-Lozinsk. with ivory white flowers, *P. vulgaris* s. str. with yellow flowers, *P. woronowii* Lozina-Lozinsk. with pink flowers, *P. sibthorpii* with purple flowers and even *P. abchasica* Sosn., with purple flowers (Lozina-Lozinskaya, 1933; Kolakovskij, 1985). Some characters like petiole length, calyx shape, corolla tube length and size of petal limb were believed to provide sufficient taxonomical resolution, at least in case of two-species classification (Lozina-Lozinskaya, 1933; Fedorov, 1952; Kosenko, 1970; Kolakovskij, 1985). It is worth to mention here that flower color in common primrose is heritable, and is likely coded by four two-allele genes (Marsden-Jones & Turrill, 1944; Marsalek, 1979), but the morphological diversity in accordance of these "species" was never investigated in details.

Many plant species have been examined to reveal the reasons for flower color diversity and geographical and spatial variation, but one general explanation is absent. Some of hypotheses are: trade-offs between cross- and self-fertilization (Clegg & Durbin, 2001) or between pollinators and herbivores (Irwin et al., 2003; Irwin & Strauss, 2005); heterogeneity of pollinator preferences (Jones & Reithel, 2001; Wolfe, 2001) which may

coincide with altitude gradient (Arnold et al., 2009); lack of phenotype integration of flower color (Frey, 2007) or the integration of flower color characters in various adaptive complexes (Frias et al., 1975; Horowitz, 1976; Hannan, 1981; Wolfe, 1993; Jewell et al., 1994; Warren & Mackenzie, 2001; Yang & Guo, 2005); frequency-dependent selection (Gigord et al., 2001; Barrett et al., 2004; Torang et al., 2006); genetic drift (Wright, 1943; Rafinski, 1979) and/or natural selection (Mogford, 1974; Schemske & Bierzychudek, 2001; Bradshaw & Schemske, 2003; Whibley et al., 2006; Matsumura et al., 2009).

From the first glance, latter two hypotheses seem to be the most appropriate in our case: there are two color forms of *Primula vulgaris* s.l. on the Caucasian Black Sea coast, and transitional zone occurs where these two forms could meet after historical separation and subsequent divergence due to selection and/or genetic drift. However, since we cannot exclude other hypotheses (Glotov & Arnautova, 1981; Shipunov & Buntman, 2001), the geospatial nature of *Primula* color polymorphism should be revealed first. Several preliminary investigations (Lozina-Lozinskaya, 1933; Richards, 2003) lead to the conclusion that the transitional zone is narrow and abrupt. This is not common in flower color polymorphism studies mentioned above, and should be checked. The hypothesis that light-colored flowers are prevalent on higher altitudes also needs examination. Lastly, morphological distinction between “species” segregated on the basis of flower color should also be clarified.

Materials and Methods

To reveal the patterns of geographic transition between different color forms, we sampled populations (topographically isolated groups of plants) of *Primula vulgaris* s.l. on almost every 10 km along the Black Sea coast between Novorossiysk and Pitsunda (Fig. 1 and Table 1). In six cases, we sampled populations with similar positions along the coast, but with different distances from the seashore and/or altitude (above the sea level). In total, 1502 plants from 71 populations were studied. One locality was investigated three times in 1997 (no. 57, cf. Table 1), 2003 (no. 44), and 2006 (no. 402) to test the invariability of flower colors proportions in one population.

Since flower color was the principal subject of our study, we introduced a color scale representing the most frequent primrose colors. This scale consists of 9 different colors (Table 2), and all the field-workers were instructed to choose the closest one. Older flowers often become darker; therefore only youngest, recently opened flowers were used for observations. Metrical representation of the colors used in statistical calculations has been done in hybrid color space of hue value (from HSB color model), lightness value (from Lab model) and magenta percent (from CMYK model); this method is similar to already used in zoology (Aguar, 2005).

Eight morphological characters believed to be distinguishable among *vulgaris* and *sibthorpii* (Lozina-Lozinskaya, 1933; Fedorov, 1952 and 1973; Kosenko, 1970; Kolakovskij, 1985) were measured (see Table 2). On each plant, we checked one biggest leaf and one flower, both for morphology and for color. We did not measure the length of pedicel because this character is age-dependent (our observations). Sampling bias was minimized by investigating multiple plants from each population. In addition to morphological measurements, we recorded geographical coordinates, altitude, and slope orientation. In seven cases, we measured pH of soil humus and ground litter.

Since all variables were significantly deviated from normality (Shapiro-Wilk test for normality p-value $\ll 0.05$ for all characters), relations between geographical/spatial characteristics (distance along the coast from Novorossiysk, distance from the shore, and altitude) and different color attributes were elucidated with non-parametric Spearman rank correlations and Kruskal-Wallis tests (non-parametric analog of one-way ANOVA). We also calculated Shannon diversity indices for flower color in populations.

In the analysis of flower color variation, the investigated populations were divided into two groups, the “coastal” (located less than four kilometers from the seashore, on Fig. 1, these populations are positioned on the seashore line) and the “mountain” (situated more than four kilometers from the seashore) populations. Coastal populations usually grow on lower altitudes (with median values were 90 m and 240 m a.s.l.).

Four different classifications of *P. vulgaris* s.l. based on morphological characters (first 8 characters from Table 2) were tested by the “randomForest” classifier (Breiman, 2001) with subsequent calculation of misclassification errors rates. We tested: (1) classification by two species according to Fedorov (1952, 1973) and Kosenko (1970); (2) by four species (Lozina-Lozinskaya, 1933); (3) by plants before transitional zone vs. plants after transitional zone, and (4) by populations with $< 50\%$ of light-colored (white and shades of yellow) flowers vs. populations with $> 50\%$ of light flowers. In addition, principal component analysis (PCA) with same scaled variables was used to determine visual quality of first classification. Since the practice of species delimitation in a studied group often employs flower colors, we also tested the correlation between morphological characters and color traits.

All statistical calculations and graphs were made in the R environment and language (R Development Core Team, 2007).

Results

The proportion of flowers with different colors did not vary significantly in one population between different years. Kruskal-Wallis test for the Kodosh population showed no significant differences between color characteristics in three different years ($\chi^2_{1.5422, df 2}, p = 0.4625$). The similar result was obtained from the test of equality of proportions (proportion of light flowers: $\chi^2_{1.1657, df 2}, p = 0.5583$)

Analysis of the relationships between the studied color traits (i.e. hue, lightness and magenta percent) resulted in high correlation coefficients (Spearman $\rho > 0.8$, $p < 0.05$); that makes them almost equal for the subsequent analyses. All color values (mean values for populations and values for individual plants) had bimodal distributions on histograms, best visible in the distribution of light flowers proportion per population (Fig. 2). Color traits were not related with pH values (Kruskal-Wallis $\chi^2_{5, df 5} p > 0.41$ for both humus and ground litter) and slope orientation (Kruskal-Wallis $\chi^2_{5.73, df 8} p > 0.67$). None of color traits was significantly related with any morphological character. The proportion of short-style plants in the population does not correspond with color traits (Kruskal-Wallis $\chi^2_{33.3, df 41} p > 0.79$).

The search for patterns of color variability revealed a clear pattern of color change along the coastal northwest-southeast direction (from Novorossiysk to Pitsunda). Light (yellow

and white) flowers were observed in northwestern populations, while different tints of red and violet (dark flowers) dominated in the south-east. The only exceptions found were the four populations in the vicinity of city Gagry (population no. 320-322 and 327; 249-254 km from Novorossiysk) where all plants had pure white and light yellow flowers. In that region, mountains come very close to the coast, and plants from observed populations grew in three isolated coastal valleys. We considered data from Gagry as outliers and did not use them in most calculations.

The revealed patterns of geographical variability of color changes were even clearer if coastal and mountain populations considered separately. Absolute values of correlation coefficients between distance along the coast and the color traits were equal to 0.31-0.38 for all populations and to 0.50-0.63 for coastal and mountain populations, taken separately (all correlations were significant with p -values < 0.05). Results of Kruskal-Wallis tests in both groups of populations were also significant (Table 3).

The “speed” of color changes along the coast was not constant, as it was shown by graphical analysis of spatial distribution of three color traits for coastal populations (Fig. 3). For each of the color trait, we found transitional zones with substantially higher relative “speeds” of changes from northwestern “light” populations to southeastern populations with diverse dark flowers. Geographical positions of the transitional zones did not vary reliably between different color traits, it was located approximately between 130 and 190 km from Novorossiysk (between Dederkoy and Dagomys) for coastal populations. For mountain populations, the transitional zone was shifted: it lied between 160 and 220 km, between Chemitokvadzhe and Khosta (Fig. 3, d). The Shannon index for flower color diversity in coastal populations also revealed the site where diversity increased (Fig. 4), but it was less expressed and wider than the transitional zone for individual color traits.

We found that plants aggregated by flower color in different ways could not be distinguished by single character or even by any combination of the investigated morphological characters. All tested classifications returned high misclassification errors, with more than 40% of individuals classified in “wrong” groups (Table 4). In addition, principal component analysis of morphological data (first three components accumulate 34.9, 26.5 and 13.6% of variation, respectively; characters loadings were almost equally distributed among components) did not coincide with the most accepted two-species classification (see above): on the graphs, convex hulls were highly intersected in all combinations of dimensions (Fig. 5).

For populations at equal positions along the coast, we did not observe the uniform dependence of flower color on the distance from the shore and altitude (Table 3). In two cases above the transitional zone (Novomikhajlovskij and Tuapse), there were no meaningful correlations. In three cases inside the transitional zone, we found several significant correlations between altitude, from the one side, and lightness and magenta values, from the other side (positive and negative, respectively). In the case of the Pitsunda populations (below the transitional zone), correlations were less significant and had the opposite sign: for example, correlation of magenta percent and altitude became positive. In all cases, correlations with the distance from the seashore were lower than correlations with altitude.

Discussion

Geographical analyses of color distribution in coastal and mountain populations suggest a relatively narrow and abrupt (60 km) transitional zone where populations with a substantial mix of light and dark flower colors are prevalent. For mountain populations, this zone is shifted southward for approximately 30 km. Inside a transitional zone, we observed clear correlation between proportion of light-colored flowers and altitude.

Along the coastal line, climate characteristics are changing constantly but smoothly towards warmer and more humid conditions along the coast, whereas the landscape remains constant (Berg, 1952). Therefore the best explanation for the presence of transitional zone of this kind would be an introgression between previously isolated color morphs, similarly to the case of *Antirrhinum majus* in Pyrenees (Whibley et al., 2006). However, the “mountain shift,” as well as significant color differences between populations from various altitudes inside the transitional zone, provide putative evidence of the selection towards lighter colors for colder (higher or more northern) regions. Since studied local conditions (namely, slope orientation and soil pH) most likely do not coincide with color traits, it is better to suppose that the presumed selection is due to changes in pollinator fauna, analogous to the case of *Rhododendron* (Stevens, 1976) and *Cirsium palustre* (Mogford, 1971). Greater diversity of pollinators in warmer regions may facilitate color polymorphism (Jones & Reithel, 2001; Wolfe, 2001) in southern populations, where the diversity is substantially higher, even outside the transitional zone. In addition, we cannot exclude the possibility that our color morphs exhibit different flower odors (Gaskett et al., 2005).

It is also worth to mention that in Holocene coastal line of Black Sea underwent dramatic changes, mostly because of rapid and intense changes of sea level (Arslanov et al., 2007; Connor et al., 2007; Martin et al., 2007). Therefore, the history of coastal zone is radically different from nearby mountains and may also contribute to the observed difference in flower colors. Some indirect support for this view is the finding of pure white-flowered populations in the Gagry area, and some red-color “outliers” near Tuapse (crosses and distant dots on Fig. 3, a). These findings suggest that neutral factors like genetic drift may provide another contribution to the observed patterns. In all, we believe that no sole hypothesis mentioned in the Introduction is fully responsible for color polymorphism of Caucasian primroses; this is a result of a joint influence of several factors. The coincidence of factors may also explain the relatively narrow geographical distribution of polymorphic populations.

Some studies of color polymorphism demonstrated association of color traits with heterostyly (Wolfe, 2001). However, we did not find any relation between these two polymorphisms of primroses; this could be an additional argument for the recent origin of their color polymorphism.

The absence of correlations between morphological characters and color, the lack of support for all the tested classifications, and lack of resolution in PCA do not allow us to accept color forms as morphologically different. The taxonomic practice (Mast et al., 2001; Richards, 2003; Compton et al., 2004) suggests that characters other than flower color should be used for species (or even subspecies) separation. Consequently, we believe that despite of the inheritance of color, there is no reason to accept these two

color forms (i.e. “*P. vulgaris* ssp. *vulgaris*” and “*P. vulgaris* ssp. *sibthorpii*”) as subspecies at least until some reliable morphological (or independent molecular) characters will be discovered.

There is another primrose with color polymorphism, *P. vulgaris* subsp. *heterochroma* (Stapf) Smith & Forrest, distributed mostly in the southern Caspian region (Azerbaijan and Iran). Most authors noted high color polymorphism (Smith & Fletcher, 1948; Fedorov, 1952; Richards, 2003) of this form, along with some morphological differences (e.g., the pubescence of leaves). Detailed studies of subsp. *heterochroma* are necessary to find out if our conclusions about origin and taxonomic meaning of color polymorphism may be expanded to this region.

We are grateful to all the members of the spring field practices (headed by S. Glagolev) of Moscow South-West High School who collected most material in the field, and personally, to E. Sysovskaya and P. Buntman, who assisted on the initial stages of data analysis, and to P. Petrov and A. Mushegian for their valuable comments on the manuscript.

References

- Aguiar A.P. (2005) An accurate procedure to describe colors in taxonomic works, with an example from Ichneumonidae (Hymenoptera). *Zootaxa*. 1008: 31-38.
- Arnold S. E. J., Savolainen V., Chittka L. (2009). Flower colours along an alpine altitude gradient, seen through the eyes of fly and bee pollinators. *Arthropod-Plant Interactions*. 3: 27-43.
- Arslanov Kh. A., Dolukhanov P. M., Gei N. A. (2007) Climate, Black Sea levels and human settlements in Caucasus littoral 50,000-9000 BP. *Quaternary International*. 167-168: 121-127.
- Barrett S. C. H., Harder L. D., Cole W. W. (2004) Correlated evolution of floral morphology and mating-type frequencies in a sexual polymorphic plant. *Evolution*. 58: 964-975.
- Berg L. S. (1952) Geographical zones of the Soviet Union. 3rd ed. Volume 2. OGIZ, Moscow [in Russian].
- Boyd M., Silvertown J., Tucker C. (1990) Population ecology of heterostyle and homostyle *Primula vulgaris*: Growth, survival and reproduction in field populations. *Journal of Ecology*: 78: 799-813.
- Bradshaw H. D. Jr., Schemske D. W. (2003) Allele substitution at a flower color locus produces a pollinator shift in monkeyflowers. *Nature*. 426: 176-178.
- Breiman L. (2001) Random Forests. *Machine Learning* 45: 5-32.
- Clegg M. T., Durbin M. L. (2001) Flower color variation: A model for the experimental study of evolution. *Proceedings of the National Academy of Sciences, USA*. 97: 7016-7023.
- Compton J. A., Clennett J. C. B., Culham A. (2004) Nomenclature in the dock. Overclassification leads to instability: a case study in the horticulturally important genus *Cyclamen* (Myrsinaceae). *Botanical Journal of the Linnean Society*. 146: 339-349.
- Connor S. E., Thomas I., Kvavadze E. V. (2007) A 5600-yr history of changing vegetation, sea levels and human impacts from the Black Sea coast of Georgia. *The Holocene*. 17: 25-36.
- Fedorov An. A. (1952) *Primula* L. In: Flora of USSR. Volume 18. Nauka, Moscow-Leningrad, pp. 111-202 [in Russian].
- Fedorov An. A. (1973) *Primula* L. In: Flora of Armenia. Volume 6. Izdatelstvo Akademii Nauk Arm. SSR, Yerevan, pp. 22-32 [in Russian and Armenian].
- Frey F. M. (2007) Phenotypic integration and the potential for independent color evolution in a polymorphic spring ephemeral. *American Journal of Botany*. 94: 437-444.
- Frias D. L., Godoy R., Iturra P., Koref-Santibanez S., Navarro J., Pacheco N., Stebbins G. L. (1975) Polymorphism and geographic variation of flower color in Chilean populations of *Eschscholzia californica*. *Plant Systematics and Evolution*. 123: 85-198.
- Gaskett A. C., Conti E., Schiestl F. P. (2005) Floral odor variation in two heterostylous species of *Primula*. *Journal of Chemical Ecology*. 31: 1223-1228.
- Gigord L. D. B., Macnair M. R., Smithson A. (2001) Negative frequency-dependent selection maintains a dramatic flower color polymorphism in the rewardless orchid *Dactylorhiza sambucina* (L.) Soo. *Proceedings of the National Academy of Sciences, USA*. 98: 6253-6255.
- Glotov N. V., Arnautova G. N. (1981) Variability of flower color in natural populations of *Primula sibthorpii* Hoffm. In: Botanical and genetical resources of Dagestan flora. Dagestan Botanical Garden, Mahachkala, pp. 81-88 [in Russian].
- Guggisberg A., Mansion G., Kelso S., Conti E. (2006) Evolution of biogeographic patterns, ploidy levels, and breeding systems in a diploid-polyploid species complex of *Primula*. *New Phytologist*. 171: 617-632.
- Hannan G. L. (1981) Flower color polymorphism and pollination biology of *Platystemon californicus*

- Benth. (Papaveraceae). *American Journal of Botany*. 68: 233-243.
- Horowitz A. (1976) Edaphic factors and flower color distribution in the Anemoneae (Ranunculaceae). *Plant Systematics and Evolution*. 126: 239-242.
- Irwin R. E., Strauss S. Y. (2005) Flower color microevolution in wild radish: evolutionary response to pollinator-mediated selection. *The American Naturalist*. 165: 225-237.
- Irwin R. E., Strauss S. Y., Storz S., Emerson A., Guibert G. (2003) The role of herbivores in the maintenance of a flower color polymorphism in wild radish. *Ecology*. 84: 1733-1743.
- Jewell J., McKee J., Richards A. J. (1994) The keel color polymorphism in *Lotus corniculatus* L.: differences in internal flower temperatures. *New Phytologist*. 128: 363-368.
- Jones N. K., Reithel J. S. (2001) Pollinator-mediated selection on a flower color polymorphism in experimental populations of *Antirrhinum* (Scrophulariaceae). *American Journal of Botany*. 88: 447-454.
- Kalman K., Medvegy A., Mihalik E. (2004) Pattern of the floral variation in the hybrid zone of two distylous *Primula* species. *Flora*. 199: 218-227.
- Kalman K., Medvegy A., Penzes Zs., Mihalik E. (2007) Morph-specific variation of floral traits associated with reciprocal herkogamy in natural populations of *Primula vulgaris* and *Primula veris*. *Plant Systematics and Evolution*. 268: 15-27.
- Karlsson M. G. (2002) Flower formation in *Primula vulgaris* is affected by temperature, photoperiod and daily light integral. *Scientia Horticulturae*. 95: 99-110.
- Kolakovskij A. A. (1985) *Primula* L. In: *Flora of Abkhazia*. 2nd ed. Volume 3. Metsiniereba, Tbilisi, pp. 138-143 [in Russian and Georgian].
- Kosenko I. S. (1970) Identification keys for higher plants of northwest Transcaucasia and Ciscaucasia. Nauka, Moscow [in Russian].
- Lozina-Lozinskaya A. S. (1933) *Primula acaulis* (L.) Hill. systematics and culture. *Bull. Acad. Sci. USSR. Matem. and Nat. Sci.* 293-307 [in Russian, English abstract].
- Marsalek, L. (1979) Dedicnost barev kvetu u *Primula acaulis*. *Zahradnictvo*. 4: 355 [in Czech].
- Marsden-Jones E. M., Turrill W. B. (1944) Experiments on color and heterostyly in the primrose, *Primula vulgaris* Huds. *New Phytologist*. 43: 130-134.
- Martin R. E., Leorri E., McLaughlin P. P. (2007) Holocene sea level and climate change in the Black Sea: Multiple marine incursions related to freshwater discharge events. *Quaternary International*. 167-168: 61-72.
- Mast A. R., Kelso S., Richards A. J., Lang D. J., Feller D. M. S., Conti E. (2001) Phylogenetic relationships in *Primula* L. and related genera (Primulaceae) based on noncoding chloroplast DNA. *International Journal of Plant Sciences*. 162: 1381-1400.
- Matsumura S., Yokoyama J., Fukuda T., Masayuki M. (2009). Origin of the disjunct distribution of flower colour polymorphism within *Limonium wrightii* (Plumbaginaceae) in the Ryukyu Archipelago. *Biological Journal of the Linnean Society*. 97:709-717.
- Mogford D. J. (1974). Flower colour polymorphism in *Cirsium palustre*. *Heredity*. 33: 241-256.
- R Development Core Team. (2007) **R**: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: <http://www.R-project.org>.
- Rafinski J. N. (1979) Geographic variability of flower color in *Crocus scepusiensis* (Iridaceae). *Plant Systematics and Evolution*. 131: 107-125.
- Richards A. J. (2003) *Primula*. 2nd ed. Timber Press, Portland, Oregon, USA.
- Schemske D. W., Bierzychudek P. (2001) Perspective: Evolution of flower color in the desert annual *Linanthus parryae*: Wright revisited. *Evolution*. 55: 1269-1282.

- Shipunov A. B., Buntman P. S. (2001) The altitude and flower color in natural populations *Primula acaulis* (L.) L. s.l. of western Transcaucasia. In: V All-Russian population seminar. Kazan' State University, Kazan', pp. 111-113 [in Russian].
- Smith W. W., Fletcher H. R. (1948) The genus *Primula*: section *Vernales*. Transactions of the Royal Society of Edinburgh. 34: 402-468.
- Stevens P. F. (1976) The altitudinal and geographical distributions of flower types in *Rhododendron* section *Vireya*, especially the Papuan species, and their significance. Botanical Journal of Linnean Society. 72: 1-33
- Torang P., Ehrlen J., Agren J. (2006) Facilitation in an insect-pollinated herb with a floral display dimorphism. Ecology. 87: 2113-2117.
- Valentine D. H. (1947) Studies in British Primulas. I. Hybridization between primrose and oxlip (*Primula vulgaris* Huds. and *P. elatior* Schreb.). New Phytologist. 46: 229-253.
- Valentine D. H. (1948) Studies in British Primulas. II. Ecology and taxonomy of primrose and oxlip (*Primula vulgaris* Huds. and *P. elatior* Schreb.). New Phytologist. 47: 111-130.
- Valentine D. H. (1955) Studies in British Primulas. IV. Hybridization between *Primula vulgaris* Huds. and *P. veris* L. New Phytologist. 54: 70-80.
- Venables W. N., Ripley B. D. (2002) Modern applied statistics with S. 4th ed. Springer, New York.
- Warren J., Mackenzie S. (2001) Why are all color combinations not equally represented as flower-color polymorphisms? New Phytologist. 151: 237-241.
- Whale D. M. (1984) Habitat requirements in *Primula* species. New Phytologist. 97: 665-679.
- Whibley A. C., Langlade N. B., Andalo C., Hanna A. I., Bangham A., Thebaud C., Coen E. (2006) Evolutionary paths underlying flower color variation in *Antirrhinum*. Science. 313: 963-966.
- Wolfe L. M. (1993) Reproductive consequences of a flower color polymorphism in *Hydrophyllum appendiculatum*. American Midland Naturalist. 129: 405-408.
- Wolfe L. M. (2001) Associations among multiple floral polymorphisms in *Linum pubescens* (Linaceae), a heterostylous plant. International Journal of Plant Sciences. 162: 335-342.
- Wright S. (1943) An analysis of local variability of flower color in *Linanthus parryae*. Genetics. 28: 139-156.
- Yang Ch., Guo Y. (2005) Floral evolution: Beyond traditional viewpoint of pollinator-mediated floral design. Chinese Science Bulletin. 50: 2413-2417.
- Zernov A. S. (2006) Flora of North-Western Caucasus. KMK, Moscow [in Russian].

Table 1. List of studied *P. vulgaris* s.l. populations.

Pop. no	Number of plants measured	Distance from Novorossiysk, km, cf. Fig. 1	Geographic origin	Date	Latitude (N)	Longitude (E)	Altitude, m	Yellow*	Light yellow*	White*	Light pink*	Pink*	Dark pink*	Pink-violet*	Blue-violet*	Purple*
501	2	52	Stream Krasnaya Schel, 3 km upper its mouth	03.05.2005	44.4371	38.2878	40	0	100	0	0	0	0	0	0	0
504	13	62	Western slope of mtn. Oblego	06.05.2005	44.5536	38.4784	400	8	77	15	0	0	0	0	0	0
505	20	62	Mtn. Oblego	06.05.2005	44.5541	38.4893	720	5	85	10	0	0	0	0	0	0
603	20	70	Vil. Arhipovo-Osipovka	06.03.2007	44.3714	38.5242	40	0	85	15	0	0	0	0	0	0
2	9	89	In the vicinity of vil. Dzhubga, mtn. Shkolnaja	25.03.2001	44.3196	38.7229	50	0	78	22	0	0	0	0	0	0
71	25	93	In the vicinity of vil. Lermontovo	07.03.2004	44.3106	38.7740	20	16	56	28	0	0	0	0	0	0
606	21	96	2 km to the north from vil. Plyaho	07.03.2007	44.2947	38.8414	100	0	24	76	0	0	0	0	0	0
605	25	98	1.5 km to the north-west from vil. Novomihajlovskij	07.03.2007	44.2675	38.8478	80	0	28	68	0	4	0	0	0	0
604	21	99	Vil. Novomihajlovskij	07.03.2007	44.2581	38.8458	60	0	9	81	5	0	5	0	0	0
73	22	110	In the vicinity of vil. Olginka	07.03.2004	44.2007	38.8946	40	0	15	55	5	10	0	5	10	0
72	25	116	In the vicinity of vil. Tyumenskij	07.03.2004	44.1835	38.9743	80	0	4	64	28	4	0	0	0	0
1	8	120	In the vicinity of vil. Agoi	25.03.2001	44.1690	39.0408	400	0	13	87	0	0	0	0	0	0
17	30	120	In the vicinity of vil. Agui-Shapsug	12.03.2002	44.2036	39.0892	62	0	17	83	0	0	0	0	0	0
18	22	120	In the vicinity of vil. Agui-Shapsug	12.03.2002	44.2031	39.0886	66	0	32	68	0	0	0	0	0	0
74	25	121	In the vicinity of vil. Nebug	08.03.2004	44.1825	39.0140	40	0	0	60	20	12	4	4	0	0
57	69	124	Cape Kodosh	25.03.1997	44.1164	39.0325	60	0	0	7	0	45	17	0	30	1
44	20	124	Cape Kodosh	16.03.2003	44.1164	39.0325	60	0	0	0	1	7	12	0	0	0
402	24	124	Cape Kodosh	07.03.2006	44.1164	39.0325	60	0	0	8	0	25	38	17	0	13
407	20	124	Cape Kodosh	09.03.2006	44.1241	39.0398	100	0	10	0	15	35	35	5	0	0
609	20	124	1 km to the north from vil. Prigorodnyij	05.03.2007	44.1317	39.1161	260	0	40	60	0	0	0	0	0	0
15	4	130	In the vicinity of city Tuapse	11.03.2002	44.1149	39.1258	50	0	100	0	0	0	0	0	0	0
16	10	130	In the vicinity of city Tuapse	11.03.2002	44.1147	39.1262	50	20	50	30	0	0	0	0	0	0
610	25	133	1.5 km to the east from vil. Shepsi	06.03.2007	44.0425	39.1711	140	0	0	88	8	4	0	0	0	0
75	25	142	In the vicinity of vil. Magri	08.03.2004	44.0370	39.1647	140	0	4	52	8	0	8	0	24	4
76	25	142	Upper part of unnamed river between riv. Shuyuk and riv. Magri	08.03.2004	44.0357	39.1757	140	0	12	52	8	0	8	0	20	0
401	21	142	2 km to the south-east from vil. Kirpichnyij	08.03.2006	44.1524	39.2288	240	0	86	14	0	0	0	0	0	0

Pop. no	Number of plants measured	Distance from Novorossiysk, km, cf. Fig. 1	Geographic origin	Date	Latitude (N)	Longitude (E)	Altitude, m	Yellow*	Light yellow*	White*	Light pink*	Pink*	Dark pink*	Pink-violet*	Blue-violet*	Purple*
602	21	142	2 km to the north from vil. Golubaya Dacha	05.03.2007	43.9936	39.2528	40	0	0	90	0	0	10	0	0	0
601	23	143	Vil. Golubaya Dacha	05.03.2007	43.9778	39.2497	120	0	0	83	0	4	13	0	0	0
303	20	153	Vil. Tikhonovka	07.03.2005	43.9652	39.2912	180	0	45	35	10	10	0	0	0	0
302	26	154	1.5 km to the south-west from vil. Mamedova Shchel	07.03.2005	43.9641	39.3022	330	0	19	81	0	0	0	0	0	0
304	20	154	In the vicinity of vil. Vodopadnyij	07.03.2005	43.9661	39.2574	40	0	50	25	5	5	0	5	0	10
305	21	154	In the vicinity of vil. Vodopadnyij	07.03.2005	43.9662	39.2572	40	0	58	14	0	14	14	0	0	0
306	20	154	In the vicinity of vil. Vodopadnyij	07.03.2005	43.9670	39.2593	65	0	45	10	20	15	5	5	0	0
307	20	154	0.5 km to the west from vil. Muhortova Polyana	07.03.2005	43.9708	39.2630	250	0	0	95	0	5	0	0	0	0
308	23	154	1 km to the south-east from vil. Vodopadnyij	07.03.2005	43.9746	39.2622	110	0	9	70	4	4	9	4	0	0
301	21	155	In the vicinity of vil. Mamedova Shchel	07.03.2005	43.9519	39.3096	140	0	5	95	0	0	0	0	0	0
77	25	160	In the vicinity of vil. Chemitokvadzhe	09.03.2004	43.8433	39.4257	80	0	0	4	16	20	44	8	4	4
78	25	160	In the vicinity of vil. Chemitokvadzhe	09.03.2004	43.8458	39.4315	230	0	0	4	0	0	0	12	76	8
79	25	160	In the vicinity of vil. Chemitokvadzhe	09.03.2004	43.8430	39.4405	40	0	0	8	0	4	4	8	56	20
309	20	164	1 km to the south-east vil. Soloniki	08.03.2005	43.8782	39.3723	20	0	0	0	0	20	55	20	0	5
310	14	164	1 km to the south-east vil. Soloniki	08.03.2005	43.8802	39.3773	40	0	0	0	0	7	64	7	0	22
311	20	164	1.5 km to the south-east vil. Soloniki	08.03.2005	43.8824	39.3830	150	0	0	10	0	15	40	20	0	15
416	18	170	Mountain range Yakornyj	09.03.2005	43.7930	39.5203	200	0	0	28	11	17	44	0	0	0
417	20	170	Mountain range Yakornyj	09.03.2005	43.8085	39.5460	300	0	0	0	5	30	40	0	0	25
418	20	170	Mountain range Yakornyj	10.03.2005	43.8203	39.5628	380	0	20	60	0	10	10	0	0	0
414	21	171	Vil. Verhneyakornaya Shchel	09.03.2005	43.7835	39.5137	120	0	0	0	0	24	33	19	10	14
415	20	171	In the vicinity of vil. Verhneyakornaya Shchel	09.03.2005	43.7878	39.5128	200	0	0	25	0	15	30	10	0	20
419	20	171	River Shahe, 3 km downstream from vil. Otradnoe	10.03.2005	43.8305	39.5742	60	0	5	60	5	10	15	5	0	0
420	20	172	River Shahe, near mouth of riv. Bzych	11.03.2005	43.8090	39.5897	140	0	0	95	0	0	5	0	0	0
312	21	176	Vil. Vardane	08.03.2005	43.7362	39.5604	60	0	0	0	5	5	33	14	5	38
313	15	177	In the vicinity of vil. Vardane	08.03.2005	43.7380	39.5651	80	0	0	0	0	0	46	27	0	27
314	19	177	Mountain range between riv. Vardane and riv. Hobza	08.03.2005	43.7377	39.5676	120	0	0	11	5	11	47	26	0	0
80	25	180	In the vicinity of vil. Uch-Dere	10.03.2004	43.6673	39.6260	20	0	0	0	0	4	0	20	60	16

Pop. no	Number of plants measured	Distance from Novorossiysk, km, cf. Fig. 1	Geographic origin	Date	Latitude (N)	Longitude (E)	Altitude, m	Yellow*	Light yellow*	White*	Light pink*	Pink*	Dark pink*	Pink-violet*	Blue-violet*	Purple*
91	21	180	Mtn. Khuko, near the lake Khuko	07.05.2004	43.9335	39.8037	1700	0	100	0	0	0	0	0	0	0
92	20	180	Mtn. Khuko, southern slope	08.05.2004	43.9308	39.8192	1700	0	100	0	0	0	0	0	0	0
422	12	180	Vil. Uch-Dere	12.03.2005	43.6701	39.6236	100	0	0	0	8	0	42	33	17	0
13	49	191	2 km to the south from vil. Baranovka	14.03.2002	43.7348	39.7118	150	0	0	33	10	31	26	0	0	0
14	52	191	2 km to the south from vil. Baranovka	14.03.2002	43.7346	39.7125	150	0	0	19	12	34	27	0	6	2
607	25	200	Vil. Verhnerazdolnoe	08.03.2007	43.6322	39.7814	400	0	0	0	4	8	32	32	0	24
608	20	202	2 km to the east from vil. Verhnematsestinskij	08.03.2007	43.6361	39.8042	420	0	5	35	20	35	5	0	0	0
81	25	210	In the vicinity of vil. Matsesta	11.03.2004	43.5554	39.8243	160	0	0	0	16	44	0	20	16	4
404	20	210	In the vicinity of vil. Matsesta	07.03.2006	43.5554	39.8243	160	0	0	0	0	5	85	10	0	0
50	19	217	In the vicinity of vil. Khosta	27.02.2003	43.5378	39.8628	340	0	0	16	37	21	26	0	0	0
421	20	219	Vil. Kudepsta	12.03.2005	43.4943	39.9038	20	0	5	0	0	25	40	5	0	25
320	7	254	In the vicinity of city Gagry, shore of stream Dzhava-Kvara	10.03.2005	43.3382	40.2273	50	0	0	100	0	0	0	0	0	0
321	10	254	In the vicinity of city Gagry, shore of stream Dzhava-Kvara	10.03.2005	43.3418	40.2303	60	0	0	100	0	0	0	0	0	0
322	20	255	In the vicinity of city Gagry, near stream Dzhava-Kvara	10.03.2005	43.3335	40.2108	120	0	0	100	0	0	0	0	0	0
327	21	256	City Gagra	12.03.2005	43.2938	40.2668	150	0	14	86	0	0	0	0	0	0
323	20	275	8 km to the south-east from cape Pitsunda	11.03.2005	43.1638	40.4174	5	0	0	0	0	10	15	30	20	25
324	15	275	8 km to the south-east from cape Pitsunda	11.03.2005	43.1655	40.4264	200	0	0	0	0	0	6	47	0	47
325	21	275	Valley of riv. Agaraki, 3 km upper its mouth	11.03.2005	43.1906	40.4242	180	0	0	0	0	0	10	33	0	57
326	20	275	1 km to the south from vil. Agaraki	11.03.2005	43.2139	40.4084	150	0	0	0	0	0	5	40	5	50

* This is a proportion (total number of the investigated plants in the population was taken as 100%). See Table 2 for the description of colors.

Table 2. Morphological characters of *P. vulgaris* s.l. studied.

Length of calyx	mm					
Length of calyx teeth	mm					
Length of corolla tube	mm					
Length of petal limb	mm					
Width of petal limb	mm					
Style	1 – short, 2 – long					
Length of maximal leaf blade	mm					
Length of petiole of maximal leaf	mm					
Flower color (lightness, hue and magenta components were taken from Lab, HSB and CMYK models, respectively)	Name	RGB value, hexadecimal	Lightness, decimal	Hue, degrees	Magenta, %	Group
	Yellow	#FFEA00	90	55	4	Light
	Light yellow	#FFFFBF	100	60	0	Light
	White	#FFFFFF	100	0	0	Light
	Light pink	#FFCCFB	85	305	22	Dark
	Pink	#D9ADD5	75	305	38	Dark
	Dark pink	#BF8FBB	65	305	56	Dark
	Pink-violet	#E291F2	70	290	47	Dark
	Blue-violet	#B39DF2	65	255	40	Dark
	Purple	#CC33B2	50	310	88	Dark

Table 3. Significance of relationships between position along the coast (NWSE), distance from the shore (SEA) and altitude (ALT), from one side, and flower color (magenta value), from other side. All data, except proportions and diversity indices, are for individual plants. Significant results with p-values <0.05 are marked in **bold**. Cases 3-7 (from Novomikhajlovskij and below) are localities where both coastal and mountain populations co-occur.

Cases	Distance from Novorossiysk (NWSE), km: mean and range	Distance from the coast (SEA), km: mean and range	Altitude (ALT), m: mean and range	Spearman ρ	Kruskall-Wallis χ^2 and degrees of freedom
Coastal (937 plants, 46 populations)	145.5 (50.8-273.5)	1.5 (0.0-4.0)	90 (5-400)	NWSE: 0.6185	612.2481, df = 45
Mountain (427 plants, 19 populations)	173.5 (56.0-269.0)	8.5 (5.0-31.3)	240 (50-1700)	NWSE: 0.6028	287.9215, df = 18
Novomikhajlovskij (67 plants)	98.5 (96.5-98.9)	2.7 (2.2-4.0)	80 (60-100)	ALT: -0.1822 SEA: -0.1822	2.213, df = 2 2.213, df = 2
Tuapse	120 (119.5-	0	48	ALT: 0.0419	19.8343, df = 5

(119 plants)	120.2)		(7-100)	SEA: n/a	n/a
Vodopadnyj (104 plants)	144.3 (144.1- 144.4)	0.5 (0.5-0.8)	65 (40-250)	ALT: -0.1747 SEA: -0.2252	7.3508, df = 3 5.2231, df = 1
Soloniki (54 plants)	157.7 (157.6- 158.0)	0.5 (0.1-1.0)	40 (20-150)	ALT: -0.003 SEA: -0.0003	3.7928, df = 2 3.7928, df = 2
Vardane (133 plants)	173.5 (172.6- 179.5)	3.5 (1.0-7.9)	200 (60-380)	ALT: -0.4293 SEA: -0.4218	43.4981, df = 5 40.9581, df = 5
Pitsunda (76 plants)	271.6 (268.9- 273.5)	2.8 (0.0-5.6)	150 (5-200)	ALT: 0.2414 SEA: 0.2303	7.3095, df = 3 7.3095, df = 3

Table 4. Misclassification errors rates between four tested *a priori* classifications and the results of “randomForest” classifier.

Classification	Misclassification error rate, %
Two species (Fedorov, 1954 and 1973 and other authors)	42.9%
Four species (Lozina-Lozinskaya, 1933)	70.4%
Plants before transitional zone vs. plants after transitional zone	54.1%
Populations with < 50% of light flowers vs. populations with > 50% of light flowers	40.1%

Figure legends

Figure 1. Map of the studied region (northwestern Transcaucasia). Circles designate locations of measured *P. vulgaris* s.l. populations (in cases of dense locations, one circle substitutes several populations) and the fraction of dark flowers in all population from given location. “Coastal” populations are positioned on the seashore line. The ruler below indicates distance along the Black Sea coast (measured from Novorossiysk). Double black line on the ruler indicates inclusive transitional zone from Figure 4 (129 to 222 km).

Figure 2. Histogram of distribution of light flowers proportion (see Table 2) among the all studied (coastal and mountain) populations.

Figure 3. The “speed” of color changes along the coast. Plots (a)-(c) represent coastal populations; plot (d) represents mountain populations (with distance from coast > 4 km). Dotted lines are linear model approximations; solid lines are the loess (local polynomial regression fitting: Venables & Ripley, 2002) approximations. Gray shades designate the position of transitional zones. On (a), the crosses in the bottom right corner belong to the Gagry “outliers” (see explanations in the text), these populations have not been shown on (b)-(d) plots. Mean population values of color characteristics were used on all plots.

Figure 4. Spatial pattern for the Shannon diversity index calculated for flower color in coastal populations. Dotted line is linear model approximation; solid line is loess (local polynomial regression fitting) approximation. Gray shades designate the position of transitional zone.

Figure 5. The plot of two first principal components from the PCA of all metric morphological characters against the classification by two “species,” *P. vulgaris* s. str. (“v”) and *P. sibthorpii* (“s”). Convex hulls mark borders of clouds of points that belong to each “species.”

Full names and addresses of authors:

- Alexey Shipunov (corresponding author): Minot State University, 500 University Ave W, Minot ND 58707, USA, e-mail: dactylorhiza@gmail.com
- Yana Kosenko: Moscow State University, Biological Department, Botanical Gardens, Prospect Mira 26, Moscow, Russian Federation, 129090, e-mail: dimorphotheca11@pisem.net
- Polina Volkova: Moscow State University, Biological Department, Vorobyevy Gory, Moscow, Russian Federation, 119899, e-mail: avolkov@orc.ru.









