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# Phenotypic diversity and landscape genetics of *Eristalis tenax* in a spatially heterogeneous environment, Durmitor Mountain (Montenegro)

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The study of the spatial distribution of phenotypic and genetic diversity of pollinators has conservation implications since pollination is a key ecosystem function and a basis for the maintenance of biodiversity. The impact of landscape heterogeneity on the population structure of the important hoverfly pollinator, *Eristalis tenax* (Diptera, Syrphidae), was investigated. Allele frequencies at allozyme loci, wing traits (size and shape) and abdominal colour pattern were compared using samples from eight locations in the Durmitor National Park, Montenegro. These locations covered a broad range of altitudes and vegetation structures, from deciduous and coniferous forests to alpine meadows. From the conservation point of view, we investigated to what extent the localities in the Durmitor mountain range are connected. Results indicated a lack of population structure in the study area. A genetic clustering analyses based on Bayesian model revealed no resolution among samples, coinciding with $F_{ST}$ estimates. Weak genetic differentiation was accompanied by wing size and shape similarity. In addition, there was an overlap between the levels of abdominal colour variation among samples supporting the negative association between gene flow and phenotypic divergence in *E. tenax*. We conclude that the surrounding landscape is of no relevance to the species movement capabilities. The availability of many sites for *E. tenax* and its strong dispersal capacity might make the study region a more or less continuous habitat for this species. Thus, our results may contribute to understanding the potential extent to which *E. tenax* can facilitate gene flow among isolated plant populations on Mt. Durmitor.

## Introduction

Understanding the response of population structure to spatial heterogeneity in habitat structure is of basic interest to ecology (Pulliam 1988, Hanski 1999), but it is broadly applicable to conservation, planning, and remediation (Debinski & Holt 2000, Cabeza & Moilanen 2003). However, examining the role that landscapes play in dispersal and gene flow can reveal much

about the effect of environmental variation on the distribution of genetic variation in natural populations (Stevens *et al*. 2006, Wang *et al*. 2009). Predictors of a species distribution are the amount of potential barriers to dispersal (Van Dyck & Baguette 2005), as well as the amount of available habitat in the surrounding landscape (e.g. Binzenhöfer *et al*. 2005). Thus, accounting for landscape heterogeneity and the precise distribution of features in a landscape can contribute to critical insights into our understanding of gene flow and population structure (Spear *et al*. 2005, Giordano *et al*. 2007). Indeed, gene flow can be restricted even in highly mobile insect species, e.g. by physical barriers on mountains (Loxdale & Lushai 2001). Furthermore, studying gene flow gives us better insight into genetic structure of populations since it significantly affects population genetic differentiation in the majority of animal species in nature (e.g. Bohonak 1999, Mun *et al*. 1999), and plays an important role in evolution both as a constraint (by preventing local adaptation) and as an aid by promoting the spread of beneficial alleles (Slatkin 1987).

For insects, however, species-specific responses at different spatial landscape scales are little understood (but *see* e.g. Roland & Taylor 1997). It is well documented that environmental variables, including altitude, ambient temperature, wind speed, relative humidity and light intensity, and demographic features of plant species, such as population size are closely interrelated with available number and behaviour of pollinators (McCall & Primack 1992, Mitchell *et al*. 2004, Zhu & Lou 2010). Contrary to more generalist species with higher dispersal capacity, a habitat specialist with poor dispersal abilities might be strongly affected by fragmentation of its habitat (Louy *et al*. 2007). Even for a single species of pollinator, individuals may show considerable heterogeneity in pattern of movement across a landscape depending upon their learning processes (e.g. Ohashi *et al*. 2007, Ohashi & Thomson 2009). Earlier studies showed that landscape structure has strong effects on species richness of solitary bees and on plant–pollinator interactions (Steffan-Dewenter *et al*. 2001, 2002, Steffan-Dewenter & Kuhn 2003).

Among insects, bees constitute perhaps the most important group in terms of pollination efficiency and diversity of plant species pollinated. Among flies, hoverflies (Diptera: Syrphidae) are one of the most important groups of pollinators (e.g. Zych 2007, Jauker & Wolters 2008, Rader *et al*. 2009). Because of their differing dispersal modes and resource requirements as compared to bees, hoverflies may play an important role in maintaining pollination services in landscapes unsuitable for bee species (Jauker *et al*. 2009, 2012). In addition, it has been previously reported that hoverflies have the capacity to transport pollen further than honeybees (Rader *et al*. 2011). Although there are some reports that hoverflies as pollinators have clear flower preferences (Fründ *et al*. 2010), most hoverfly species are often regarded as generalist in flower use. Therefore, we choose to study the dronefly, *Eristalis tenax* (Syrphidae, Eristalinae), a widespread hoverfly with potential for long-distance dispersal and migratory behaviour. Indeed, observations in mountain areas indicated that individuals of this species are able to cross high mountain ranges like the Alps (Aubert & Goeldlin de Tiefenau 1981). Under natural conditions, for example, in the case of the absence of bees on small islands, *E. tenax* proved to be invaluable for the viability and the long-term persistence of the local flora (Pérez-Bañón *et al*. 2003), and plays an invaluable conservation and perhaps evolutionary role for the native plants (Pérez-Bañón *et al*. 2007). In addition, *E. tenax* seems to be particularly important pollinator in unfavourable ecological conditions at high altitudes (Zoller *et al*. 2002, Zhu & Lou 2010). Since this hoverfly appears to be relatively efficient long-distance pollen disperser, more attention should be drawn to the migratory paths of *E. tenax* (Pérez-Bañón *et al*. 2003) and what is shaping them.

The purpose of this study was to investigate spatial distribution of phenotypic and genetic diversity of *E. tenax*, a taxon widely distributed in a range of landscapes of Mt. Durmitor, Montenegro. Mountain ranges are among most species-rich landscapes in Europe and, therefore, of special conservation interest. However, while mountain tops are often well conserved, their lower slopes are generally poorly protected (Sergio & Pedrini 2007). Therefore, our sampling locations covered a broad range of

altitudes and vegetation structures, from deciduous and coniferous forests to alpine meadows. Since conectivity studies of widespread species indicated that gene flow among populations was not limited by geographical distance *per se*, but strongly dependent on the type and share of suitable and unsuitable habitat (Lange *et al*. 2012), genetic differentiation might be registered even in widespread and abundant species (such as *E. tenax*) under specific landscape conditions (Lange *et al*. 2010). Thus, from the conservation point of view, we investigated to what extents the localities in Durmitor mountain range are connected. This study might also indicate if pollen could be dispersed by droneflies over complex landscapes.

Since the abundance pattern and dispersal behaviour of species influence the connectivity of local populations, exchange rates can be indirectly measured by genetic and phenotypic differentiation among samples. One way to obtain insight into the factors shaping the genetic structure of populations is to combine genetic data with information on landscape characteristics, referred to as landscape genetics (Manel *et al*. 2003). Therefore, we examined spatial diversity across heterogeneous landscape using allele frequencies at 17 allozyme loci, wing traits (size and shape) and abdominal colour pattern in eight samples of *E. tenax* from Mt. Durmitor, Montenegro. Having a great potential for investigating processes such as gene flow, migration or dispersal (Holderegger *et al*. 2006), allozymes allow us to empirically test the functional relevance of spatial indices used in landscape ecology (Holderegger *et al*. 2006). Connectivity estimates should have the potential to be altered according to evolutionary changes, when and where dispersal behaviour of individuals is affected by landscape configuration (Baguette & Van Dyck 2007). We also used wing geometric morphometrics because it was widely employed as a complementary tool to genetic data to get information on population structure. Indeed, using wing morphologies to determine population structure has been encouraged by some authors who consider this method to be a low-cost alternative for the preliminary estimation of population structure (Dujardin 2008, Morais *et al*. 2010). In addition, adaptive importance of wing traits has been previously proposed. For instance, wing size is usually associated with body size, and thus potentially linked to a number of fitness components (Reeve *et al*. 2000), but wing shape is related to flight ability which might also influence fitness (Kölliker-Ott *et al*. 2003). Wing size and shape are thought to have different genetic properties, and therefore different patterns of evolution (Bithner-Mathé & Klaczko 1999, Andrade *et al*. 2009). As compared with wing size, wing shape has proved to be under tighter genetic control, and to be less sensitive to environmental changes (Bitner-Mathé & Klaczko 1999, Matta & Bitner-Mathé 2004). Still, there are many genes involved in regulating wing size and shape in insects (Mezey & Houle 2005, Mezey *et al*. 2005), although many still remain unidentified (De Celis 2003). Thus, geometric morphometrics is tempting as a candidate population marker because it is informative about current or very recent population events, and it contains information on genetic variation (Dujardin 2008). Finally, the third marker used herewith is abdomen colour pattern which can additionally indicate the associations between gene flow and phenotypic divergence in *E. tenax* samples, which has been previously suggested by Heal (1982). An explanation for variation in abdominal colour pattern was proposed concerning the thermoregulatory requirements of the fly (Heal 1981, Holloway 1993). Namely, breeding studies of *E. tenax* have previously shown that environmental variables influence final adult colour patterns in that low temperatures during pupation result, on average, in darker abdominal patterns (Dušek & Láska 1974, Heal 1989, Ottenheim *et al*. 1996). Furthermore, some *E. tenax* abdominal phenotypic plasticity may represent a degree of protection from predators as a result of its resemblance to the honey bee (e.g. Heal 1982). To outwit the influence of temperature, *E. tenax* carries genetic polymorphism that contributes substantially to colour variation. A major *Ap* (abdominal pattern) locus has been identified with alleles for light and dark colour patterns, and heritability estimates showed further genetic variation other than at the *Ap* locus (Heal 1979). Finally, bearing in mind that a system ecology approach is recommended in order to quantify the structural

importance of Diptera to pollination networks (Ssymank *et al*. 2008), we studied three different markers using both population- and individual-based approaches in a comparative way to gain insight into spatial patterns of population connectivity of *E. tenax*.

## Material and methods

### Study species and landscape characterisation

The study species *Eristalis tenax* is an anthropophilic and almost ubiquitous one. Adults are active with two or three generations from early spring to late autumn (Gilbert 1986). The adults hibernate and can even be found on particularly warm January and February days. They feed on nectar from flowers, but the larvae feed on rotting organic material in stagnant water and are known as rat-tailed maggots. When *E. tenax* insects visit flowers, the hairy legs, thorax and abdomen catch pollen grains and transfer them to the next visited flower (Zhu & Lou 2010), and the efficiency of *E. tenax* as a pollinator may be enhanced by the presence of many palynophilic hairs on the insect body (Pérez-Bañón *et al*. 2003). In this way, *E. tenax* was observed between 2700 m and 3200 m flying from cushion to cushion and promoting outcrossing in alpine plant *Eritrichium nanum* (Boraginaceae) (Zoller *et al*. 2002). Under the hostile weather conditions, *E. tenax* is also the principal pollinator of a high-mountain perennial plant, *Rhodiola dumulosa* (Crassulaceae) (Zhu & Lou 2010). During the tripping process, the pollen grains are mainly widespread on the anterior half of the ventral surface of the thorax, where they can remain for several days (Holloway 1976). *Eristalis tenax* is a locally abundant species and, although specific studies on dispersion and migration of this species are lacking, its wide altitudinal and geographical distribution, high mobility and ability to live in distinct habitat types suggest at least moderate dispersion rates.

The flies were sampled from 21 to 25 July of the year 2009 while they were feeding on pollen or nectar at flowers or resting on bare ground or vegetation. Allozyme variation and differences in wing traits and abdominal colour were analysed using the same individuals. Because of the sexual dimorphism this species exhibits (Francuski *et al*. 2011), wing traits and abdominal colour pattern analyses were performed separately for males and females.

The Durmitor National Park lies high in the southeastern Dinaric Alps. It ranges in altitude from about 450 m to 2522 m a.s.l. Because of its location and altitudinal range, the Park has both Mediterranean and alpine microclimates resulting in an exceptional range of species and habitats. Durmitor lower slopes are generally poorly protected and characterized by intensively managed agricultural areas and patchily distributed fragments of forest and semi-natural grassland habitats, while mountain tops are relatively well conserved. We selected this structurally complex landscape as a study region and eight sampling locations including three mountain peaks (Veliki Štuoc, Savin Kuk and Prutaš, Table 1 and Fig. 1) with similar elevation (above 2000 m a.s.l.). By using high altitude mountain peaks, we tried to find out if they may act as impermeable barriers in this geographical area to highly mobile species such as *E. tenax*. More detailed information on locations and sampling points are summarized in Table 1.

### Methods

#### Population structure

A total of 123 specimens (Table 1) were included in the allozyme analysis by vertical polyacrylamide gel electrophoresis (PAGE). Allozyme polymorphism was studied at 17 different loci: aconitate hydratase (4.2.1.3. ACO; two loci: *Aco-1*, *Aco-2*), aspartate amino transferase (2.6.1.1. AAT; *Aat*), esterase (E.C. 3.1.1.? EST; *Est-2, Est-4*), fumarate hydratase (4.2.1.2. FUM; *Fum*), glycerol 3-phosphate dehydrogenase (1.1.1.8. GPD; *Gpd-2*), hexokinase (2.7.1.1. HK; *Hk-2*), isocitrate dehydrogenase (1.1.1.42. IDH; two loci: *Idh-1*, *Idh-2*), malate dehydrogenase (1.1.1.37. MDH; *Mdh-2*), malic enzyme (1.1.1.40. ME; *Me*), phosphoglucomutase (2.7.5.1. PGM; *Pgm*), sorbitol dehydrogenase (1.1.1.14. SDH; *Sdh-1*, *Sdh-2*), superoxide dis-

mutase (1.15.1.1. SOD; *Sod-1*, *Sod-2*). The tris-borate-EDTA buffer system (pH 8.9) was used to assay EST, FUM, HK, ME, PGM and SOD, while Tris-citrate buffer system (pH 7.1) was used in analysis of AAT, ACO, GPD, IDH, MDH and SDH. Details on the buffer systems and staining procedures are given in Munstermann (1979) (EST, FUM, GPD, GPI, HK, MDH, ME, PGM, SOD) and Pasteur *et al*. (1988) (AAT, AO). The duration of the electrophoretic run at 90 mA (135–220 V) was 3–4 hrs. Specimens from all samples were run concurrently on all gels to facilitate comparisons of electrophoretic mobility. Loci and alleles were numbered with respect to order of increasing anodal migration following Francuski *et al*. (2011). Four of the 17 loci analysed were polymorphic: *Aat*, *Est-2*, *Est-4* and *Me*.

Parameters of the population genetic structure were calculated using the computer program BIOSYS-2 (Swofford & Selander 1989).

We used two Bayesian clustering methods to investigate the spatial genetic structure of the dronefly in the sampled region. Firstly, we analysed the population genetic structure using a Bayesian model executed in the STRUCTURE

**Table 1.** Sampling points and sample sizes of *Eristalis tenax* used for geometric morphometrics, abdomen colour pattern and allozyme analyses.

| Sampling locations* | Characteristics and land cover of sampling locations | Sampling points and altitude | Allozyme analysis | Wing traits and abdomen colour pattern analyses | |
|---|---|---|---|---|---|
| | | | | male | female |
| 1. Veliki Štuoc | Softly-sloped mountain peak, at an altitude of about 2000 m a.s.l. forest zone ends with dwarf pine and juniper shrubs; highest parts are covered with karsts and calcareous grassland | Grassland, top of the mountain peak; 2200 m a.s.l. | 15 | 11 | 11 |
| 2. Lake Crno | Largest Lake Durmitor, surrounded by mixed coniferous forest including spruce, fir, beech, black pine and maple | Lakeshore forest edge; 1422 m a.s.l. | 15 | 23 | 19 |
| 3. Plateau | Grassland open space with lakes, lot of creeks and water sources, mesophilic meadows and pastures | Meadow surrounded by arable fields; 1200 m a.s.l. | 13 | 22 | 9 |
| 4. Komarnica | Canyon with its a same name river surrounded by mixed forest of oriental hornbeam (*Carpinus orientalis*) and xerothermal maple (*Acer* sp.) | Forest edge; 1000 m a.s.l. | 16 | 11 | 5 |
| 5. Savin Kuk | Mountain peak covered with alpine pastures and meadows | Grassland, top of the mountain peak; 2313 m a.s.l. | 16 | 13 | 14 |
| 6. Prutaš | Mountain peak, at an altitude of about 2000 m a.s.l. forest zone ends with mugo pine and juniper shrubs, highest parts are covered by rocky subalpine and alpine meadows | Grassland, top of the mountain peak; 2393 m a.s.l. | 16 | 8 | 16 |
| 7. Lake Škrčko | Located in the center of glacial cirque valley, surrounded by low forest of mesial beech and fir around the lake | Lakeshore forest edge; 1686 m a.s.l. | 16 | 10 | 9 |
| 8. Lake Sušičko | Periodical lake; largest and full of water in spring time when glaciers melt; during summer the water disappears and stays a green grassy meadow | Meadow (bottom of the lake valley); 1140 m a.s.l. | 16 | 18 | 11 |

* Numbers as in Fig. 1.

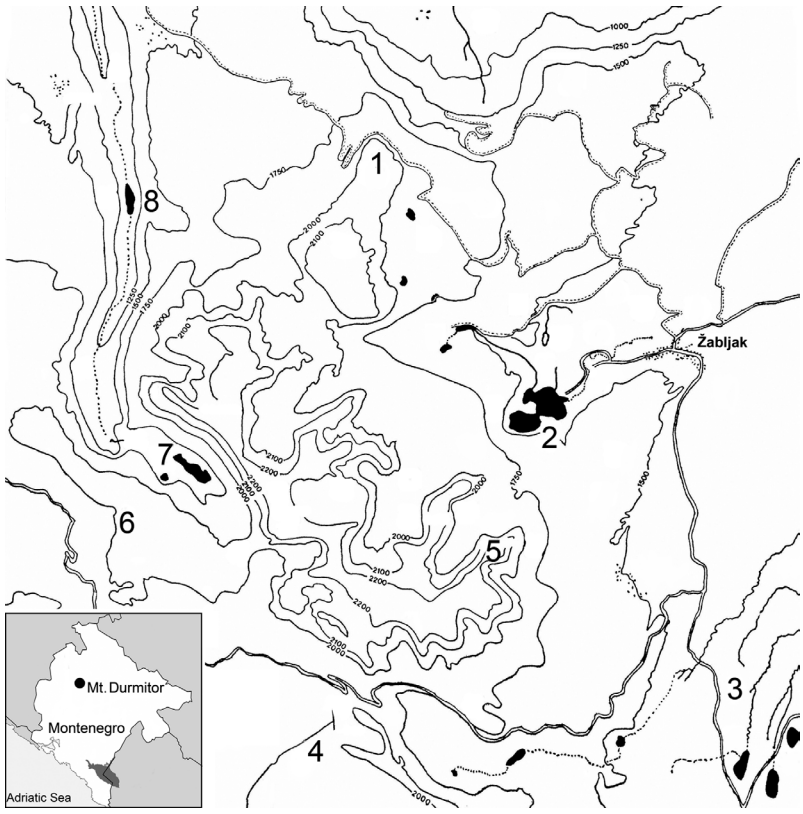


**Fig. 1.** Map of Mt. Durmitor. Geographic location of sampling locations of the study area: 1. Veliki Štuoc; 2. Lake Crno; 3. Plateau; 4. Komarnica; 5. Savin Kuk; 6. Prutaš; 7. Lake Škrčko; 8. Lake Sušičko. Black areas indicate the position of lakes.

software (Pritchard *et al*. 2000). This analysis delineates clusters of individuals as populations (*K*), based on their multilocus genotypes. In our STRUCTURE analyses, we performed runs with values of *K* set from 1 to 8, the latter of which was the number of sampling localities. Specifically, we selected the correlated allele frequencies model and the admixture model with a burn-in period of 100 000 and 100 000 MCMC (Markov chain Monte Carlo). Five runs were performed for each value of *K*, to check the consistency of the results between runs with the same *K*. We estimated the number of clusters and the assignment of individuals into clusters using two methods. First, the most likely number of clusters was estimated by determining the change in the marginal likelihood of the data Pr(*X*|*K*) when the numbers of clusters (*K*) was fixed to different values (Pritchard *et al*. 2000). We also implemented the Δ*K* method *sensu* Evanno *et al*. (2005) to detect the amount of structuring. Δ*K* is the second-order rate of change of the marginal likelihood function and takes into account both the gain in posterior probabilities over a range of *K* values and the variance between independent runs at given values of *K*. Results of all the runs were summarized using STRUCTURE HARVESTER ver. 0.6.92 (Earl & vonHoldt 2012).

Secondly, we employed the spatial Bayesian clustering method implemented in the program BAPS 5.4. (Corander *et al*. 2003, 2006), using samples with known geographical coordinates as units to be clustered. We used the "spatial clustering of groups" options (Corander *et al*. 2006), which utilises the spatial location of each individual to provide a biologically meaningful non-uniform prior distribution, without making an assumption regarding what is a biological population (Corander & Marttinen 2008). This increases the power to detect underlying population genetic structure when it exists (Corander *et al*. 2008). In the spatial clustering model, the landscape occupied by the population is divided into a 'coloured Voronoi tessellation' (Deussen *et al*. 2000), in which different colours in the tessellation represent genetically differentiated

samples. The model specifies the colouring, corresponding to an estimate of the underlying genetic population structure, jointly for all cells of the tessellation. In the present application, the spatial model was fitted using 20 replicate runs, each with the maximum number of 8 clusters (corresponding to the number of sample plots in our sample).

Analysis of molecular variance (AMOVA) was performed using ARLEQUIN ver. 3.1 (Excoffier *et al*. 2005) to examine the distribution of genetic variation within and among populations (samples). Using genotypic data of four polymorphic loci, one group of populations and no within-individual level, we tested all sampled individuals with < 5% of missing values. Ten thousand permutations were used to determine the significance of variance components.

We then employed Wright's *F*-statistics (Wright 1951, Conner & Hartl 2004) to measure genetic differentiation among samples. Treating each sample as a separate population, we calculated genetic differentiation among samples using $F_{ST}$. The pairwise $F_{ST}$ values between eight samples were calculated with FSTAT ver. 2.9.3.2 (Goudet 2001), and the significance between each comparison pair was evaluated through 1000 permutation procedures.

Association between genetic and geographic distances was assessed by Mantel's test (Mantel 1967). Approximate linear geographical distances (measured in kilometres) between sample pairs were obtained with Google™ Earth software and prior to analysis geographic distances were ln-transformed. Estimates of genetic distances between pairwise populations were characterized as $F_{ST}/(1 - F_{ST})$ (Rousset 1997). The significance levels of associations were evaluated by permutation. This analysis was performed with the Isolation By Distance Web Service ver. 3.23 (Jensen *et al*. 2005) using a total of 10 000 permutations.

Finally, a landscape shape interpolation of genetic distances, which interpolates calculated genetic distances across the landscape and shows them graphically as heights ($z$ axis) in a graph where the base (values along $x$ and $y$ axes) represents the geographic space, was obtained using the software Alleles in Space (AIS) (Miller 2005). We used several different distance weighting values ($a$ = 0.25–2), grid sizes, and both raw and residual genetic distances to make sure that interpretations were not sensitive to these parameters. Across the genetic landscape, the peaks and troughs indicate high and low genetic distances between individuals respectively.

## Geometric morphometrics

The right wings of 210 specimens (116 males and 94 females) were mounted in Hoyer's medium and then digital images of them were taken with a Leica DFC320 camera connected to a Leica MZ12.5 stereomicroscope. In each wing image, a total of 16 landmarks were digitized using TpsDig ver. 1.40 (Rohlf 2004). Locations of landmarks selected for geometric morphometric analysis follow Francuski *et al*. (2011).

For comparing overall wing size between groups, we used centroid size which is an isometric estimator defined as the square root of the sum of the squared distances between the center of the configuration of landmarks and each individual landmark (Bookstein 1991). Size variation was explored by means of ANOVA and a Tukey test. To analyse variation in wing shape, a full set of shape variables ($w$ matrix; the matrix of partial warp scores; Rohlf *et al*. 1996), including both non-uniform and uniform shape components, was used for canonical variate analysis (CVA).

Both centroid size and $w$ matrix were obtained utilizing TpsRelw 1.44 (Rohlf 2006), and all statistical analyses were calculated using STATISTICA ver. 10 for Windows (StatSoft Inc.).

## Abdomen colour patterns

We scored the ventral abdomen colour pattern following Francuski *et al*. (2011), assigning specimens into arbitrary groups based on the marking size, shape and colour (yellow, orange, brown, black). Combining eight arbitrary groups of tergite 2 and seven groups of tergite 3, 23 different abdomen phenotypes were assessed. Each abdominal pattern was allocated to one of the categories and colour pattern frequencies were calculated for samples and genders. Correspond-

ence analyses were performed to examine differences in abdomen phenotype distribution among samples. We completed statistical analyses using contingency tables with columns as populations and rows as phenotype frequencies in STATISTICA ver. 10.

## Results

### Allozyme diversity

Twelve enzyme systems coded by alleles of 17 loci were assayed in eight samples of *E. tenax*. Four of the 17 loci analysed were polymorphic in all samples: *Aat*, *Est-2*, *Est-4* and *Me*. A $\chi^2$-test for deviations of observed heterozygosity ($H_o$) from expected ($H_e$) revealed that departure from the Hardy-Weinberg equilibrium for the *Aat* and *Me* loci was common in the studied samples. Significant difference was found in three samples for both *Est-2* (Veliki Štuoc, Komarnica and Lake Škrčko) and *Est-4* (Plateau, Komarnica and Savin Kuk) loci. Genotype fixation index, $F_{IS}$, indicated excess homozygosity ($F_{IS} > 0$) in all samples at variable *Aat* and *Me*, and in most samples at *Est-2* (Veliki Štuoc, Komarnica, Prutaš, lakes Škrčko and Sušičko) and *Est-4* (Plateau, Komarnica, Savin Kuk, Prutaš, Lake Škrčko). Excess heterozygosity ($F_{IS} < 0$) was estimated at *Est-2* in three samples (Lake Crno, Plateau, Savin Kuk), while reduced homozygosity was observed at *Est-4* in Veliki Štuoc, lakes Crno and Sušičko. These results were in accordance with Selander's *D* statistics.

Analysis of population genetic structure parameters showed small differences in the average frequency of observed heterozygosity ($H_o$) among Durmitor samples ($H_o$ ranged from 0.017 in Veliki Štuoc, to 0.059 in the Lake Crno sample). In addition, $H_e$ per population ranged from 0.064 to 0.108. The mean number of alleles per locus also showed no pronounced differences ($A$ = 1.3–1.4), while the frequency of polymorphic loci ($P$ = 23.5%) was equal in all samples (Table 2).

### Spatial genetic structure

The non-spatial Bayesian clustering analyses (using STRUCTURE software) revealed no

**Table 2.** Estimates of genetic structure parameters in the samples of *Eristalis tenax*.*A* = Mean number of alleles per locus; SE = standard error; *P* = Frequency of polymorphic loci based on the 0.95 criterion; $H_o$ = Average frequency of observed heterozygosity; $H_e$ = Average frequency of expected heterozygosity. Monomorphic loci in all samples: *Aco-1*, *Aco-2*, *Fum*, *Gpd-2*, *Hk-2*, *Idh-1*, *Idh-2*, *Mdh-2*, *Pgm*, *Sdh-1*, *Sdh-2*, *Sod-1*, *Sod-2*. Polimorphic loci in all samples: *Aat*, *Est-2*, *Est-4* and *Me*).

| Locus | Veliki Štuoc | Lake Crno | Plateau | Komarnica | Savin Kuk | Prutaš | Lake Škrčko | Lake Sušičko |
|---|---|---|---|---|---|---|---|---|
| *A* (SE) | 1.3 (0.1) | 1.4 (0.2) | 1.3 (0.1) | 1.4 (0.2) | 1.3 (0.1) | 1.3 (0.1) | 1.4 (0.2) | 1.4 (0.2) |
| $P_{(0.95)}$ | 23.5 | 23.5 | 23.5 | 23.5 | 23.5 | 23.5 | 23.5 | 23.5 |
| $H_o$ (SE) | 0.017 (0.017) | 0.059 (0.040) | 0.034 (0.034) | 0.023 (0.009) | 0.034 (0.034) | 0.037 (0.026) | 0.024 (0.020) | 0.031 (0.024) |
| $H_e$ (SE) | 0.084 (0.039) | 0.100 (0.046) | 0.064 (0.038) | 0.082 (0.040) | 0.087 (0.043) | 0.104 (0.047) | 0.108 (0.049) | 0.091 (0.046) |

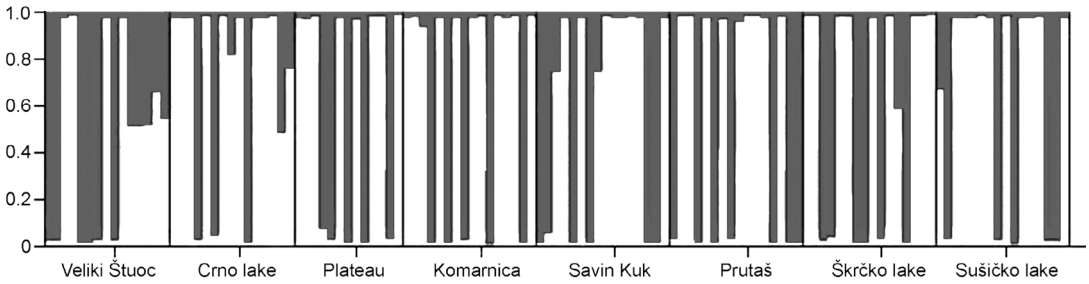


**Fig. 2.** Membership of *Eristalis tenax* individuals in a number of presumed "populations". Population clusters ($K = 2$) determined by the *a priori* Bayesian cluster method in STRUCTURE. Each vertical line represents an individual's probability of belonging to one of the $K$ clusters (represented by grey and white).

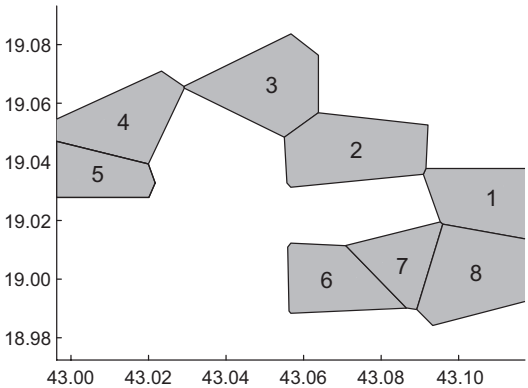


**Fig. 3.** Genetic clustering of samples in the study area inferred with the program BAPS. Identical grey of sample plots indicates homogeneous genetic composition in the study area. Names of sampling locations are given in Fig. 1 and Table 1.

resolution among samples. Across a range of potential subgroup sizes from $K$ = 1–8, there was no clear indication of clustering since all samples showed a high degree of admixture. Most probable number of clusters indicated by a declining rate of increase in Pr($X|K$) and Evanno's $\Delta K$ was two ($K = 2$). However, two genetic clusters (clusters 1 and 2) were almost evenly represented in samples, suggesting an absence of distinct genetic discontinuities of *E. tenax* samples (Fig. 2). Taking spatial information into account, BAPS gave a probability of 99.3% of there being a single genetic cluster in the study area (Fig. 3). As a result, we assume that there is no coarse partitioning of genotypes on this landscape. Moreover, AMOVA revealed no significant difference among samples with only 3.62% of the variation among populations, while a huge amount (96.38%) of the variation was partitioned within populations (Table 3). The $F_{ST}$ averaged 0.036 ($p = 0.547$) and corroborated non-significant ($p > 0.05$; Table 4) pairwise $F_{ST}$ values among samples in showing that inter-population gene flow was not negligible. In addition, we found no evidence of isolation by distance in the Mantel test ($r = 0.06$, $p = 0.597$).

The genetic landscape generated in AIS revealed that several landscape features were associated with increased or decreased inter-individual genetic distance (Figs. 1 and 4), suggesting that these features restrict or facilitate gene flow, respectively. Genetic distances peaked in the northern part of the study region (around the Veliki Štuoc peak). Other peaks are less pronounced than those in the north. Genetic distances were the lowest on the eastern edge (Lake Crno, Plateau) and associated with troughs. The same general patterns were found regardless of the choice of grid size and a distance-weighting parameter.

**Table 3.** The AMOVA results for eight *Eristalis tenax* samples from Mt. Durmitor. Fixation Index $F_{ST} = 0.036$, $p = 0.547$.

| Source of variation | d.f. | Sum of squares | Variance components | Percentage of variation |
|---|---|---|---|---|
| Among populations | 7 | 11.602 | 0.033 | 3.62 |
| Within populations | 238 | 167.542 | 0.864 | 96.38 |
| Total | 245 | 179.144 | 0.897 | |

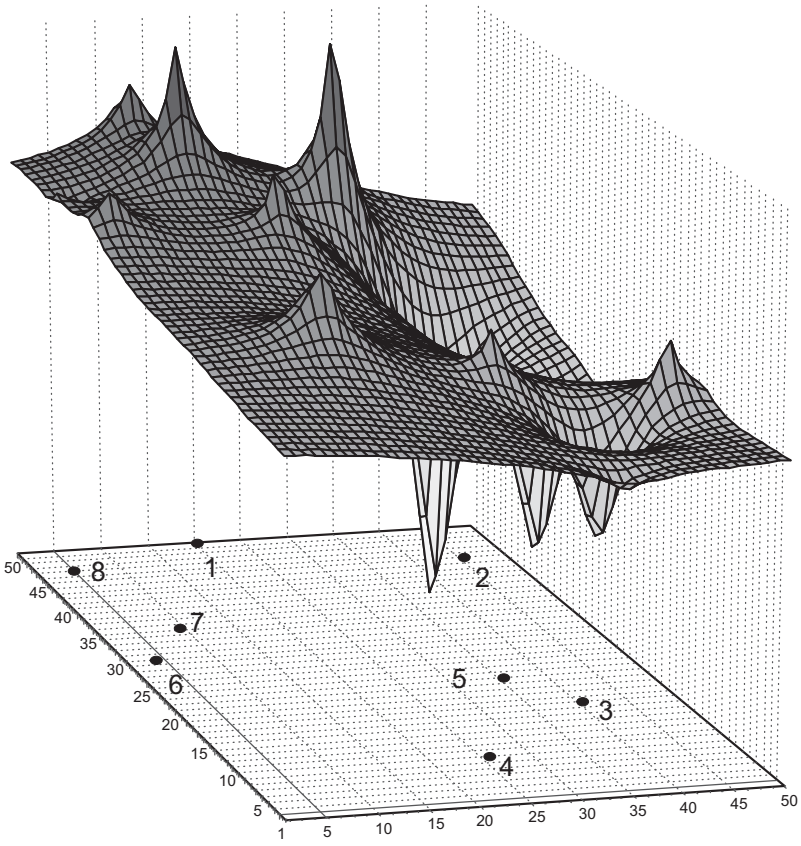


**Fig. 4.** Results of genetic landscape shape interpolation analysis for *Eristalis tenax* using a distance weighting parameter (*a*) of 1 and raw genetic distances; *x* and *y* axes correspond to geographic locations and surface plot heights reflect genetic distances. Qualitatively similar results were obtained using both raw and residual genetic distances, different grid sizes, and a range of distance weighting parameters. The positions of sampling locations in the "base" of the graph are approximate: 1. Veliki Štuoc; 2. Lake Crno; 3. Plateau; 4. Komarnica; 5. Savin Kuk; 6. Prutaš; 7. Lake Škrčko; 8. Lake Sušičko.

## Wing geometric morphometrics

*Eristalis tenax* males collected from different parts of Mt. Durmitor showed size similarity ($F_{7,13}$ = 0.21, $p$ = 0.981, all post-hoc Tukey HSD pairwise comparisons were not significant). Similarly, the CVA scatter plot (Fig. 5) showed that male samples are not well separated in the analysis. Non-significant divergence in wing shape (Wilks' $\lambda$ = 0.148, $F_{196,71}$ = 1.178, $p$ = 0.069) allowed 61% of all male individuals to be classified correctly (classification rates ranged between 46% and 80%). Similarly to males, females did not show significant differences in wing size ($F_{7,12}$ = 1.23, $p$ = 0.289, all post-hoc Tukey HSD pairwise comparisons

**Table 4.** Pairwise comparisons among eight samples of *Eristalis tenax* from Mt. Durmitor. Pairwise $F_{ST}$ values are below diagonal, and pairwise geographical distances (km) among sampling sites are shown above diagonal.

| | Veliki Štuoc | Lake Crno | Plateau | Komarnica | Savin Kuk | Prutaš | Lake Škrčko | Lake Sušičko |
|---|---|---|---|---|---|---|---|---|
| Veliki Štuoc | | 12.90 | 21.03 | 25.04 | 18.04 | 18.86 | 17.20 | 25.04 |
| Lake Crno | 0.0361 | | 15.58 | 17.01 | 2.65 | 9.26 | 6.43 | 9.47 |
| Plateau | 0.0802 | –0.0247 | | 15.96 | 13.75 | 21.50 | 20.96 | 25.11 |
| Komarnica | –0.0016 | 0.0501 | 0.0622 | | 13.19 | 14.46 | 15.04 | 20.37 |
| Savin Kuk | –0.0905 | 0.0426 | 0.0659 | 0.0129 | | 10.68 | 9.63 | 13.28 |
| Prutaš | –0.0630 | 0.0296 | 0.0618 | 0.0347 | –0.0187 | | 1.50 | 6.16 |
| Lake Škrčko | –0.0743 | 0.0049 | 0.0231 | –0.0114 | –0.0481 | –0.0570 | | 5.23 |
| Lake Sušičko | –0.0672 | –0.0027 | 0.0076 | –0.0029 | –0.0553 | 0.0030 | –0.0378 | |

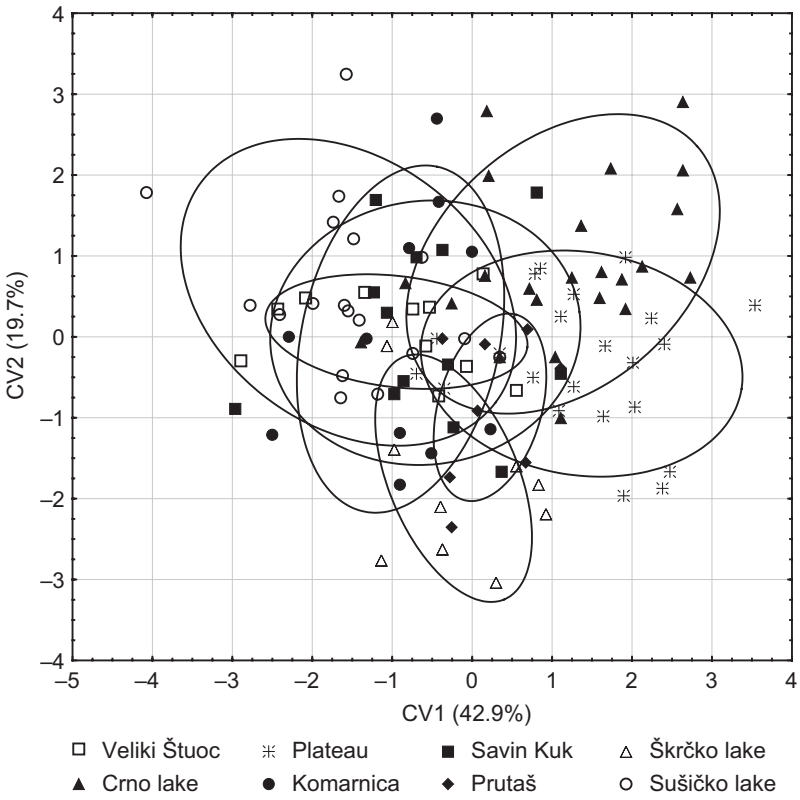


**Fig. 5.** Scatterplot of individual scores from the CVA showing wing shape differentiation among samples of male *Eristalis tenax* from the Durmitor region. The amount of variation is given in parentheses.

were not significant). In addition, CVA with collection sites as a grouping variable revealed non-significant wing shape differences among female specimens (Wilks' $\lambda = 0.118$, $F_{196,65} = 1.237$, $p = 0.058$). The scatter plot showed that there was no clearcut separation between the groups (figure not shown). Based on wing shape, 89% of flies caught at Lake Crno were correctly classified, whereas percentages of correct classification were lower for other collection sites (Lake Škrčko 69%, Prutaš 66%, Savin Kuk 64%, Plateau 62%, Lake Sušičko 60%, Komarnica 40% and Veliki Štuoc 35%).

## Abdomen colour patterns

Variation of abdomen colour and patch size in different landscapes was investigated for male and female specimens separately. Based on the colour patterns of the tergites 2 and 3, there were no pronounced spatial differences among male samples. Abdomen phenotype frequencies (correspondence analysis) revealed no differentiation among males (Total Inertia = 0.483, $\chi^2 = 205.75$, df = 189, $p = 0.193$). Light phenotype A2B2 was dominant in all samples. Tergite combination A2B7 was shared by only Prutaš/ Plateau sample pair, while phenotype A2B4 was unique for Plateau. In addition, males collected in Lake Škrčko shared one abdomen phenotype with each Lake Crno (A3B5), and Savin Kuk (A7B7) sample (Table 5).

Similarly, phenotypic divergence among spatial female samples was tested using tergites combinations and revealed no pronounced spatial differences (Total Inertia = 0.325, $\chi^2 = 128.54$, df = 133, $p = 0.593$). All female samples had common dominant pattern A1B7 (yellow A1 morph of the tergite 2 and black B7 morph of the tergite 3). Phenotypes with smaller coloured patches (A1B1, A4B1 and A4B7) also occurred frequently in collections, while orange A6B5 phenotype was unique for the Veliki Štuoc sample (Table 5).

## Discussion

### Pattern of genetic diversity is not concordant with landscape features

Herewith, weak genetic structuring indicates ongoing gene flow in *E. tenax* across sampled sites on Mt. Durmitor. Using both population- (Wright's $F_{ST}$ value) and individual-based levels (individual's genotypes), we found limited (if any) effects on genetic structuring of *E. tenax* since none of the Durmitor samples displayed a significant degree of genetic differentiation from one to another. Considered in concert, our results suggest that the studied sites where droneflies were sampled exhibit substantial connectivity. Moreover, an observed lack of isolation by geographical distance (IBD) is likely to be a result of the dispersal power of our study species. Given the continuous distribution of the species in the study area, and considering that individual hoverflies are highly dispersive, it was likely that the data set would not exhibit an IBD pattern.

Furthermore, we studied whether landscape features and habitat connectivity influenced dispersal patterns and genetic structure among natural conspecific populations as has been previously reported (Spear *et al*. 2005, Giordano *et al*. 2007). Interestingly, we found that different landscape features affect patterns of gene flow in opposite directions (although we found no evidence of distinct genetic breaks in *E. tenax*). The Veliki Štuoc peak was associated with increased genetic distance, thus suggesting a restriction of gene flow. The Savin Kuk (in the eastern part) peak, on the other hand, was associated with genetic-distance troughs, indicating high levels of gene flow. Similarly, genetic distances of the Prutaš region were not as high as in the Veliki Štuoc region. Beside similar elevation and veg-

**Table 5.** Abdominal colour pattern frequencies of *Eristalis tenax* from the Durmitor region. Abdomen colour patterns were scored following Francuski *et al.* (2011).

| Phenotype | Veliki Štuoc | Lake Crno | Plateau | Komarnica | Savin Kuk | Prutaš | Lake Škrčko | Lake Sušičko |
|---|---|---|---|---|---|---|---|---|
| **Males** | | | | | | | | |
| A2B2 | 0.46 | 0.52 | 0.22 | 0.55 | 0.15 | 0.26 | 0.20 | 0.50 |
| A2B3 | 0.09 | 0.14 | 0.22 | 0.09 | 0.15 | – | – | 0.06 |
| A2B4 | – | – | 0.09 | – | – | – | – | – |
| A2B5 | 0.09 | 0.09 | – | – | 0.15 | 0.12 | 0.20 | – |
| A2B7 | – | – | 0.09 | – | – | 0.12 | – | – |
| A3B2 | – | – | 0.05 | 0.09 | – | – | 0.10 | 0.06 |
| A3B3 | – | 0.04 | 0.05 | 0.27 | 0.15 | 0.12 | 0.10 | 0.16 |
| A3B4 | 0.09 | – | 0.09 | – | 0.08 | 0.26 | – | – |
| A3B5 | – | 0.04 | – | – | – | – | 0.10 | – |
| A4B1 | – | 0.04 | 0.09 | – | | – | – | – |
| A4B2 | 0.09 | – | – | – | 0.08 | – | – | 0.06 |
| A4B5 | 0.09 | 0.09 | – | – | – | – | – | – |
| A6B5 | – | 0.04 | 0.05 | – | – | 0.12 | 0.20 | 0.10 |
| A7B7 | – | – | – | – | 0.08 | – | 0.10 | – |
| A8B6 | 0.09 | – | 0.05 | – | 0.15 | – | – | 0.06 |
| **Females** | | | | | | | | |
| A1B1 | 0.09 | – | – | 0.20 | 0.14 | 0.06 | 0.22 | 0.18 |
| A1B6 | – | – | – | – | 0.07 | – | 0.11 | 0.09 |
| A1B7 | 0.46 | 0.53 | 0.45 | 0.60 | 0.30 | 0.38 | 0.34 | 0.55 |
| A4B1 | – | 0.05 | 0.11 | 0.20 | 0.07 | 0.12 | – | – |
| A4B2 | – | 0.11 | – | – | 0.21 | 0.12 | – | – |
| A4B6 | 0.27 | – | 0.11 | – | – | 0.06 | 0.11 | 0.09 |
| A4B7 | – | 0.10 | – | – | 0.21 | 0.26 | 0.22 | 0.09 |
| A5B7 | 0.09 | 0.05 | 0.22 | – | – | – | – | – |
| A6B5 | 0.09 | – | – | – | – | – | – | – |
| A7B7 | – | 0.16 | 0.11 | – | – | – | – | – |

etation coverage of the analysed mountain peaks, we also required information if the sites around peaks differ, and thus have potential impact on *E. tenax* population differentiation at this spatial scale. As detailed information about the habitat quality and configuration of the surrounding landscape is lacking, the study of its role is a subject for future research. Nevertheless, based on our result, we suspect that different mountain peaks have similar effects on *E. tenax* gene flow. Non-significant $F_{ST}$ estimates among samples indicate that probably none of the landscape features represent impermeable barriers to movement. Generally, complex landscapes with a high habitat diversity, a high proportion of semi-natural habitats and small mean patch area should provide a more continuous supply of nectar and pollen than structurally simple landscapes (Beekman & Ratnieks 2000, Steffan-Dewenter *et al*. 2002). In addition, the abundance pattern and dispersal behaviour of species influence the connectivity of local populations. In this context, since *E. tenax* is spread almost evenly at all altitudes and in all types of ecosystems across the study region (Šimić 1987), we assume that Mt. Durmitor with high amounts of flowering plants is attractive to, and can support a large number of the *Eristalis* flies. The availability of many habitats for *E. tenax* and its strong dispersal capacity might make the study region a more or less continuous habitat for this species, with no or only rather weak genetic differentiation. Such slight genetic structuring in diverse, high gene-flow species can be ecologically important although cannot be detected by conventional methods (Kelly *et al*. 2010). As gene flow increases, values of $F_{ST}$ become small relative to their confidence intervals (Waples 1998), making it impossible to assess subtle genetic subdivision without impractically large sample sizes (Kelly *et al*. 2010). Since most previous work on intraspecific genetic differentiation in Syrphidae has been based on mitochondrial DNA haplotype and allozyme variation and focused on longer time scales in the context of incipient speciation (e.g. Ståhls *et al*. 2008, Milankov *et al*. 2008), further examination should be directed to higher resolution markers (such as microsatellites) and to studies at smaller spatial scales (i.e., local or regional). However, our results support that *E. tenax* is capable of moving across heterogeneous landscape and thus possess sufficient movement capabilities to maintain moderate to high rates of gene flow.

## Diversity of wing traits and abdominal colour pattern are in concordance

After characterizing the genetic structure of *E. tenax*, we wanted to determine whether flies collected across the study region differed in their wing and abdominal morphology. In line with allozyme analysis, a pattern of wing size similarity was observed. Likewise, the wing shape analysis found that there was no way of separating the samples into groups. The results seem to indicate that *E. tenax* inhabiting different landscapes developed very similar wing shapes and sizes. Similar to genetic data, the lack of phenotypic structuring in wing traits is likely to be the result of high mobility of adult *E. tenax* flies. However, as long as morphometric traits have much higher environmental variance (Dujardin 2008) than genetic markers, they are not appropriate for gene flow estimation. As a consequence, they are not appropriate for estimating the level of migrants. Due to their sensitivity to diversifying selection among subpopulations or habitats, they could underestimate true levels of migration (Dujardin 2008). Nonetheless, we consider our findings important as negative associations between gene flow and phenotypic divergence are common for many taxa (e.g. King & Lawson 1995, Henry *et al*. 2010). Yet, a spatial scale of tens of kilometres may be too small to accumulate differentiation in a species as mobile as the dronefly.

A lack of differences *E. tenax* wing traits in this study were accompanied by a lack of abdominal colour differentiation. In fact, we found an overlap between the levels of abdominal colour variation among the *E. tenax* samples. With relatively small sample sizes and potentially 23 different abdomen phenotypes, variation in pattern frequencies is likely to reflect sample size. However, the distribution of dominant phenotype patterns was unaffected and differences among Durmitor samples were not obvious. Comparing females, all samples had common dominant

pattern A1B7. In males, light phenotypes A2B2, A3B3 and A3B3 were frequently found throughout the study region. In addition, it was already suspected that high mobility of adult *E. tenax* flies limits the abdomen colour differentiation between populations (Heal 1982). A number of abdomen phenotypes found in analyzed samples are in concordance with the proposed phenotypic plasticity of the abdominal colour patterns of *Eristalis* species (Heal 1982, Holloway 1993, Ottenheim *et al*. 1996). The lack of significant differences in abdominal variation of the dronefly was also observed among temporal samples of the species (Heal 1989, Francuski *et al*. 2011), indicating that seasonal phenotype changes do not affect the focal phenotype trait. Since variation in abdominal colour pattern was proposed concerning the thermoregulatory requirements of the fly (Heal 1981, Holloway 1993) and protection from predators as a result of its resemblance to the honey bee (e.g. Heal 1982), it seems reasonable to consider that pigmentation of adult specimens in our collection is influenced by the similar temperature experienced during pre-imaginal stages, or that different pupal treatments have not made a strong effect on hair colouration in *E. tenax*.

## Conservation implication

Genetic and phenotypic evidence presented here indicates moderate to high level of gene flow across the Durmitor region, suggesting that hoverfly's dispersal ability is sufficient to establish gene flow over a network of habitats involving the whole study region. Thus, our results may suggest that droneflies are able to maintain adequate pollination over the Durmitor area and have some general implications for plant–pollinator interactions and gene flow in a landscape context. From a conservation perspective, our data suggest that *E. tenax* dispersal activity presumably influence genetic connectivity of insect-pollinated plant populations and thus have relevance for conservation of local flora. In addition, the findings that high-elevation mountain ranges do not restrict dispersal of *E. tenax* may contribute to understanding the potential extent to which the species can facilitate gene flow among isolated plant populations on high Durmitor mountain peaks. Although the focus in conservation is often on endemic organisms that are at risk of decline or extinction, we agreed with Mock *et al*. (2007) that it is also important to consider organisms that may be favoured by current environmental changes, such as *E. tenax*. Actually, studies of species composition and interactions indicated that specialized, locally rare plants tend to be visited by generalized, locally abundant animals (Vázquez & Aizen 2004, Stang *et al*. 2007). Therefore, conservation biologists are concerned with pollinator diversity and the effects of pollinator extinction on the reproduction of rare plants and their populations (Kearns *et al*. 1998, Biesmeijer *et al*. 2006). Such pattern could have implications for conservation and stress the importance of preserving not only rare species, but also the more abundant ones, such as *E. tenax,* which may be key pollinators in the plant flower-visitor network (Dupont *et al*. 2003).

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