Spatial and temporal distribution of Tabanidae in the Pyrenees Mountains: the influence of altitude and landscape structure

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Abstract

In high-altitude summer pastures, horseflies (Diptera: Tabanidae) can be a serious nuisance to livestock, as well as mechanical vectors of animal diseases such as besnoitiosis, an enzootic disease in the Pyrenees. However, the activity of horseflies in mountainous environments is poorly documented. To study the seasonality and distribution of tabanids in the Pyrenees Mountains, a sampling design was set up in two valleys on opposite sides of the mountain, one north-facing and one south-facing, along high-elevation gradients and at different distances from a water body between May and October 2011. The influence of the landscape on species richness and abundance was assessed by taking into account forested and unforested areas in 200 m radii around the trapping sites. Our findings indicated that: (1) The slope, the altitude and the size of unforested patches significantly influenced community composition of tabanids. (2) Altitude had a positive or a negative effect, depending on the species. (3) Species richness and abundance were negatively correlated with large open habitats and positively correlated with patch-shape complexity. (4) Seasonal succession of the most abundant species was observed in both valleys, with a maximum of catches at the beginning of August; however, tabanid activity ended earlier in the southern valley, which was more exposed to sunlight. (5) *Philipomyia aprica*, *Tabanus bromius*, *Tabanus glaucopis* and *Hybomitra auripila* were active from 9:00 to 19:00 h (GMT + 1), with a peak of activity at midday. This paper also discusses the implications of these findings in relation to changes in horsefly distribution and their control in mountainous environments.

Keywords: Tabanidae, Pyrenees, elevation, landscape, seasonal abundance, daily activity

Introduction

The management of suckler cattle in European mountain conditions is based on long winter housing phases, imposed by the climatic conditions, followed by summer grazing on high-altitude pastures during the period of higher availability and quality of grass (Casasus *et al.*, 2002). However, in

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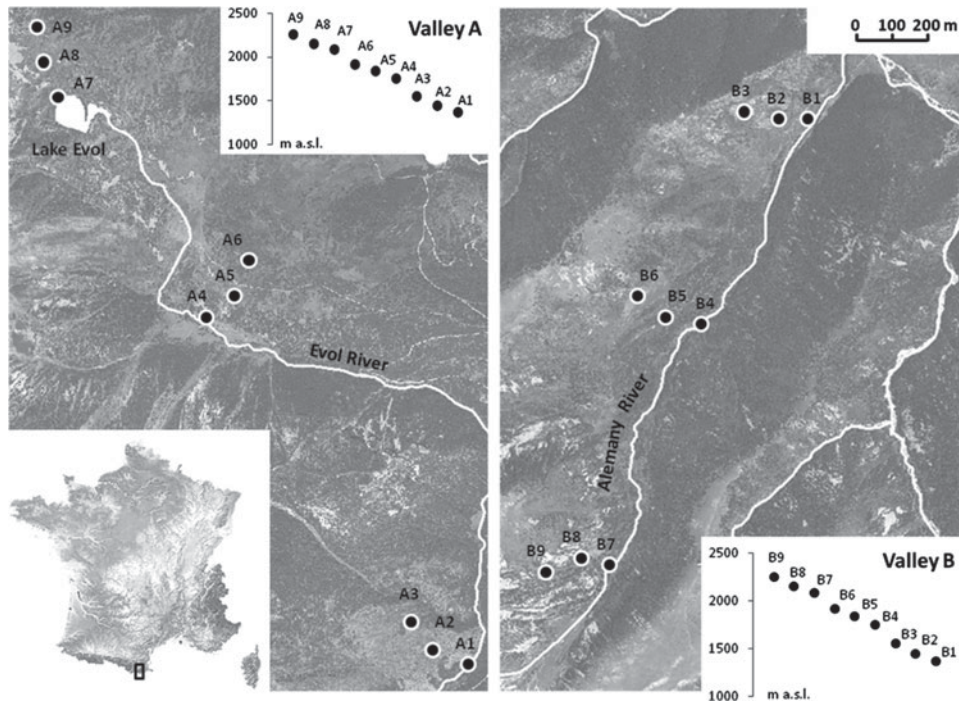E-mail: frederic.baldacchino@cefe.cnrs.fr

Fig. 1. Studied valleys (A and B) with vertical profiles of the trapping locations. Trapping locations were numbered from 1 to 9.

mountainous areas, cattle are particularly exposed to ectoparasites, especially horseflies (Diptera: Tabanidae). Horseflies are large haematophagous insects that feed on the blood of ungulates and can be a serious nuisance for livestock (Mullen & Durden, 2002). Previous studies have shown that grazing animals are likely to be particularly bothered by tabanids, and when the fly load is high, they may move to habitats with maximal exposure to wind or with mixed vegetation, for example, bushy areas, where tabanids are less active (Hughes *et al.*, 1981; Raymond & Rousseau, 1987). In addition, tabanids are mechanical vectors of pathogens such as *Besnoitia besnoiti*, a sporozoan parasite enzootic to the Pyrenees, but currently considered an emerging disease in Europe (Foil, 1989; EFSA, 2010; Jacquiet *et al.*, 2010; Gentile *et al.*, 2012). High-altitude pastures are areas with a high risk of transmission, as sick livestock can mix with healthy livestock in the herd.

The occurrence and activity of tabanids depend on the presence of the host animal, as well as on environmental parameters such as climatic conditions (temperature, humidity and wind) and landscape parameters (water, vegetation and altitude) (Chvála *et al.*, 1972; Sheppard & Wilson, 1977; Hackenberger *et al.*, 2009; Van Hennekeler *et al.*, 2011). Insect communities may be influenced by habitat variation at the scale of a patch and/or the landscape, depending on the focal taxa's body size, home range area and dispersal distances. For example, species richness and abundance of bumblebees in mountainous areas is influenced by both patch and landscape factors (Hatfield & LeBuhn, 2007).

In Europe, most ecological studies dealing with tabanids have been conducted in plains habitats, with only a few studies carried out in mountainous areas (Chvála, 1979; Hackenberger *et al.*, 2009). However, Koerner (2007) underlined the importance of altitude in ecological research, and

altitude has been virtually proven to influence the distribution of insects (McCoy, 1990; Hodkinson, 2005; Pyke *et al.*, 2011). Moreover, the richness of Tabanidae fauna in European mountains (the Alps and the Pyrenees) was shown by Leclercq (1971, 1977) who has attempted to characterize the altitudinal distribution of species.

The eastern Pyrenees are an ideal study site for evaluating the influence of environmental variables on diversity and abundance of tabanids. The climate of this region is influenced by both Mediterranean and oceanic climatic conditions (Esteban *et al.*, 2009). The transversal aspect of the main valleys in relation to the north–south axis results in two clearly contrasted slopes, one dry and one wet. Furthermore, agricultural and pastoral activities have created a structured mosaic of landscapes, modified by the expansion of forest areas over the last few decades (Gracia *et al.*, 2011). The selection of this area for our study allowed us to consider the effects of different altitudes along high-elevation gradients, the effects of distance from water bodies – which are known as breeding sites for tabanids – and the effects of landscape factors on the distribution of tabanids. Seasonality and daily activity patterns of the main species present were also recorded.

Materials and methods

Study sites

Two sampling valleys (A and B) of summer pasture grounds on opposite slopes were selected in the eastern Pyrenees, France (fig. 1). Valley A is located in the summer pastures of La Mouline, ranging from 850 to 2300 m above sea level on the southeast slope of the Madres massif (2°14′E, 42°37′N). The climate is influenced by Mediterranean and
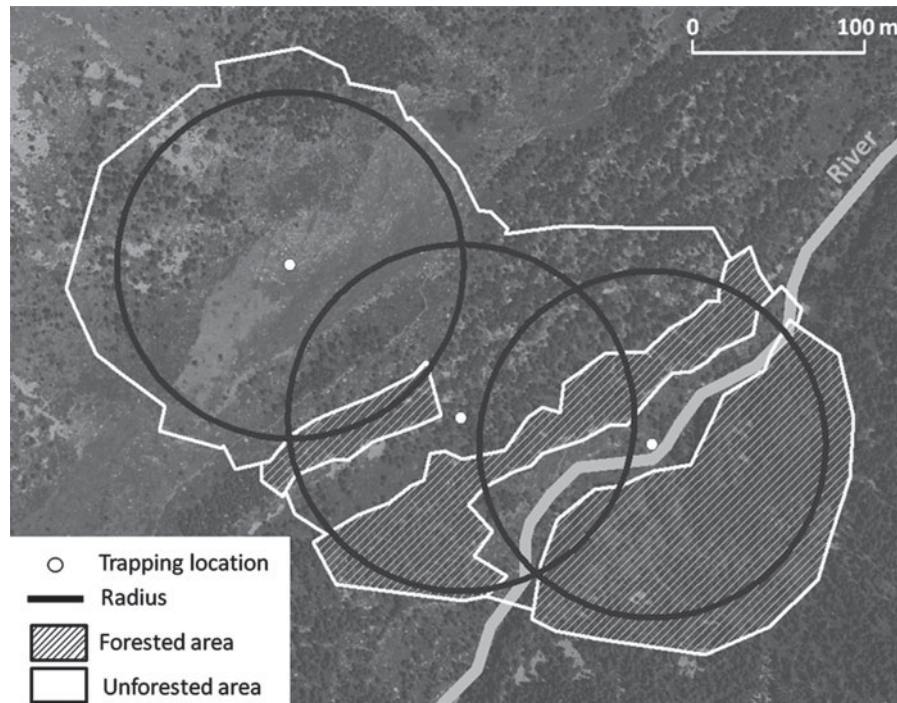
Fig. 2. Example of 200 m radii around three trapping locations. Polygon-shaped patches of forested and unforested habitats were delineated using aerial photographs.

alpine conditions, with a mean annual temperature of 10.8°C and annual rainfall of 753 mm (Nohèdes village, 1000 m a.s.l., at a distance of 4 km) (Roux *et al.*, 2011). This area is in the centre of the Madres-Coronat Natura 2000 site (FR9101473) and includes Evol Lake ('Gorg Negre') at 2083 m a.s.l. It is characterized by dense grassland (*Isoetes echinospora*) around the lake, peat bogs, forests of mountain pine (*Pinus mugo*) and granitic scree.

Valley B is located in the Mantet Natural Reserve, ranging from 1450 to 2700 m a.s.l. on the northwest slope of the Costabona massif (2°18′E, 42°28′N). The climate is influenced by both moderate Atlantic and orogenic continental conditions with a mean annual temperature of 9.5°C and annual rainfall of 855 mm (Mantet village, 1545 m a.s.l.) (Mantet Natural Reserve data, unpublished). This area is characterized by metamorphic rocks from the Mantet–Fillos rift. It is a complex mosaic of woodlands (*Pinus uncinata* and *Betula* spp.), moorland (*Cytisus oromediterraneus*), rocks, pastures and grasslands. Wetlands and aquatic habitats are poorly represented.

Sampling

Figure 1 represents the two valleys with the vertical profile of the trapping locations. To evaluate the altitudinal effect, three transects at different altitude levels were designated in each valley: low (A1–A3: 1373–1554 m, B1–B3: 1580–1732 m), medium (A4–A6: 1757–1921 m, B4–B6: 1824–1932 m) and high (A7–A9: 2087–2258 m, B7–B9: 2101–2205 m). Along each transect, three locations were fixed at an increasing distance from the nearest river (0 m, 200 m and 400 m) to evaluate the influence of the proximity of water bodies. In both valleys, the respective rivers were considered the main water

body, except for the highest altitude level of valley A, where the transect starts at the shore of the lake.

Mapping

To evaluate the influence of landscape factors, aerial photographs were analysed using ArcMap 9.3 (ESRI Inc., California, USA). Radii of 200 m were overlaid on trapping locations (fig. 2). The radii were quite large in size because tabanids are strong, fast fliers. Studies conducted on tabanid dispersal have shown that the mean daily flying distance is less than 1–2 km, although unfed tabanids may disperse over distances of more than 6 km (Sheppard & Wilson, 1976; Cooksey & Wright, 1987; Konstantinov, 1993; Barros & Foil, 2007). In each radius, polygon-shaped patches of homogeneous habitat were delineated according to the vegetation cover using aerial orthophotography from 2009 and classified into two land-cover classes: forested and unforested (fig. 2). Forested patches corresponded to conifer forests, and unforested patches corresponded to open areas such as grasslands, pastures, moors, bushes, rock, water or mixed vegetation.

Landscape metrics were calculated in each radius using the patch analyst extension for ArcGis 9.x. In each radius, forested and unforested patches were characterized by their total surface area (CA, ha), the mean patch size (MPS, ha) and their mean perimeter–area ratio (MPAR, m ha^{-1}) as an indicator of the patch-shape complexity (table 1).

Around trapping locations, the landscape was largely dominated by unforested habitat. The number of forested and unforested patches was quite similar, but the MPS was four times higher for unforested patches. Conversely, the MPAR was two times higher for forested patches.

Table 1. Landscape parameters extracted from thematic maps for each radius with the mean and standard deviation for each class (forested vs unforested).

Classification	Parameter (abbreviation)	Class	Mean	SD	Unit
Area	Class area (CA)	Forested	2.83	2.47	ha
		Unforested	9.73	2.47	ha
Patch density and size metric	Mean patch size (MPS)	Forested	1.87	1.96	ha
		Unforested	7.68	3.94	ha
Shape metric	Mean perimeter–area ratio (MPAR)	Forested	726.2	708.2	m ha^{-1}
		Unforested	373.3	508.3	m ha^{-1}

Trapping

One Nzi trap (Mihok, 2002) and one Vavoua trap (Laveissiere & Grebaut, 1990) were used at each trapping location. The distance between traps was about 10 m. The traps were made from blue and black components (SuperMaine 300 g cotton/polyester 65/35, TDV Industries, France) and polyester mosquito netting. Aged cow urine (50 ml) was used as the attractant and placed under each trap in plastic vials with a 2.5-cm aperture. The front of each Nzi trap was positioned facing west. A total of eight collections in each valley were made over the period of May to September 2011. Each collection was conducted over a continual 48-hour period. Tabanids were identified using Chvála's key (Chvála 1972). Each sampling unit (SU) consisted of the total catches obtained in a pair of Nzi-Vavoua traps during a 48-hour session. Then, all the abundance data per species from the 72 SUs (8 collections × 9 trapping locations per valley) were used to analyse the spatial and temporal distribution of tabanids.

Daily activity pattern

To investigate the daily activity pattern of tabanids, collections were made in selected locations on selected days and times. In valley A, collections were made at three locations (A3, A4 and A7) on 1 August 2011, and in valley B at five locations (B1, B2, B4, B5 and B6) on 11 August 2011 and at three locations (B7, B8 and B9) on 7 July 2012. Species were collected during six consecutive 2-hour periods from 7:00 to 19:00 h (GMT + 1). Only one Nzi trap baited with aged cow urine was used at each location.

The results took account of all trapping locations in valley A and B during the three collection days. The percentages of the total number of each species were calculated for each 2-hour period.

Statistical analyses

Differences in catches between Nzi traps and Vavoua traps were compared using the non-parametric Wilcoxon signed-rank test (*W*). Species accumulation curves for each valley were constructed using EstimateS (Colwell *et al.*, 2012). A correspondence analysis and a canonical correspondence analysis (CCA) were performed per trapping location on abundance log-transformed data using Canoco 4.5 (ter Braak & Smilauer, 2002). For the CCA, the environmental data-set consisted of a matrix of nine variables: the slope (valley A vs valley B), the altitude, the distance from the river and the three landscape metrics (CA, MPS and MPAR) calculated for forested (F) and unforested (UF) patches (table 1). During the

CCA, Monte Carlo permutations ($n = 499$) were performed in order to identify the subset of environmental variables that exerted a significant influence on tabanids distribution at $P < 0.01$. Correlations between the total number of species or the abundance of main species and (i) the altitude, (ii) the distance from the river and (iii) the landscape metrics were evaluated using Spearman's rank-order correlations coefficient (r_s) with Monte Carlo permutations ($n = 9999$). Statistical tests were performed with PAST 2.12 (Hammer *et al.*, 2001).

Results

Effectiveness of traps

A total of 2020 tabanids (2019 females and one male) belonging to 19 species in six genera (*Philipomyia*, *Tabanus*, *Hybomitra*, *Atylotus*, *Haematopota* and *Chrysops*) were captured over the period of the study (table 2). Most of the insects, including the only male, were *Philipomyia aprica* (Meigen 1820) (55.8%). Other abundant species were *Tabanus bromius* Linnaeus 1758 (13.4%), *Hybomitra auripila* (Meigen 1820) (9.8%) and *Tabanus glaucopis* Meigen 1820 (9.2%).

The Nzi traps consistently caught more tabanids than the Vavoua traps, with a total of 1824 specimens (90%): *Philipomyia* spp. (88%, $W = 36$, $P = 0.01$), *Tabanus* spp. (92%, $W = 21$, $P = 0.04$), *Hybomitra* spp. (88%, $W = 15$, $P = 0.09$) and *Atylotus* spp. (83%, $W = 12$, $P = 0.27$). Nzi traps seemed less effective for *Chrysops* spp. (63%, $W = 3$, $P = 0.6$) and *Haematopota* spp. (28%, $W = 6$, $P = 0.33$), and very few specimens of these two genera were collected.

Spatial distribution

During the study, we collected more species but less specimens in valley A ($N = 18$, $n = 731$) than in valley B ($N = 13$, $n = 1289$). Catches of the four main species were more numerous in valley B. Species accumulation curves show that the sampling effort was quite sufficient for estimating species richness in both valleys and that species richness was higher in valley A (fig. 3).

The initial correspondence analysis revealed that trapping location A7, close to the lake, was an outlier (fig. 4). Therefore, a second correspondence analysis was done without this sample. Locations in valleys A and B were separated along the first axis, while higher locations (A8, A9, B8 and B9) were clustered along the second axis. To investigate community composition in relation to environmental variables, a CCA was performed (fig. 5). The A7 sample was not included in the analysis and two samples were downweighted as they were detected as outliers with a high influence. The results of the CCA were significant, explaining 66.2% ($P = 0.002$) of tabanids

Table 2. List of collected Tabanidae species and the number of individuals of each species.

| Species	Valley A, n	Valley B, n	Total, n
P. aprica (Meigen 1820)	468	659	1127
T. bromius Linnaeus 1758	68	202	270
H. auripila (Meigen 1820)	31	166	197
T. glaucopis Meigen 1820	9	177	186
Hybomitra montana (Meigen 1820)	28	32	60
Hybomitra distinguenda (Verrall 1909)	17	16	33
Haematopota pluvialis (Linnaeus 1758)	25	6	31
Atylotus fulvus (Meigen 1804)	26	3	29
Tabanus maculicornis Zetterstedt 1842	26	1	27
Tabanus quatuornotatus Meigen 1820	4	13	17
Tabanus sudeticus Zeller 1842	3	7	10
Tabanus exclusus Pandelle 1883	8	0	8
Chrysops relictus Meigen 1820	8	0	8
Hybomitra caucasica (Enderlein 1925)	1	4	5
Haematopota scutellata (Olsufjev, Moucha & Chvála 1964)	4	0	4
Tabanus rupium (Brauer 1880)	0	3	3
Chrysops caecutiens (Linnaeus 1758)	3	0	3
Tabanus miki Brauer 1880	1	0	1
Chrysops sepulcralis (Fabricius 1794)[1]	1	0	1
Total	731	1289	2020

[1] Specimen not collected in a trap.
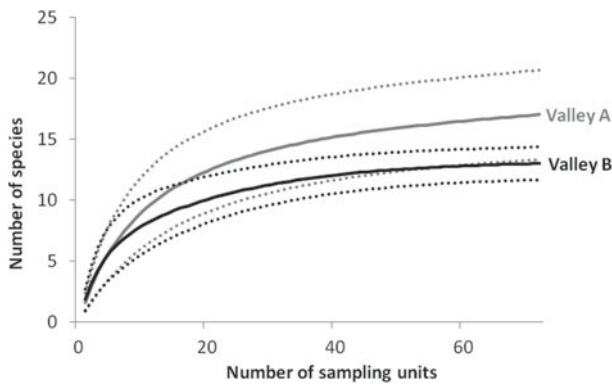
Fig. 3. Species accumulation curves for valleys A and B (each is shown with its 95% confidence interval).

distribution. The results indicated that three variables significantly influenced fauna: slope, altitude and the mean patch size of unforested patches ($P < 0.01$).

The relationships between the number of taxa or the abundance of the main species and (i) the altitude, (ii) the distance from the river and (iii) the landscape context were also assessed by Spearman's correlation analysis. All the significant results are summarized in table 3. They show that (i) no correlation occurred between the diversity of tabanids and altitude. However, the abundance of *P. aprica* and *T. bromius* were negatively correlated with altitude in valleys A and B, whereas *H. auripila* was positively correlated with altitude in valley B. (ii) Distance from the river had no perceptible effect on the catches. (iii) Species richness was positively correlated with the size of forested patches, and negatively correlated with the surface covered by unforested habitats. The shape complexity of unforested patches also had a positive effect on the number of taxa. In both valleys, the abundance of *P. aprica* was positively correlated with the shape complexity of unforested patches, but negatively correlated with the size of unforested patches. In valley B, the size of unforested patches had a negative effect on the abundance of *T. bromius* and *T. glaucopis*, and the shape complexity of unforested patches had a positive effect on the abundance of *T. bromius*.

Seasonal abundance and daily activity

In both valleys, tabanids were active from early June to early September – only a few specimens were collected in late May and late September. The largest number of individuals and species were collected in early August in valley A and in mid-August in valley B (fig. 6). There was a seasonal succession of the four main species. *H. auripila* was the first species collected in abundance, with a peak in late June in both valleys; *P. aprica* was active from late July to mid-August, with the shortest flight period in valley A; *T. bromius* had the longest flight period, from late June to mid-August, with a peak in abundance in early August in valley A, extending to mid-August in valley B. The last to appear was *T. glaucopis*, which peaked in abundance in mid-August in valley B.

Tabanids were active from 9:00 to 19:00 h (fig. 7). The diurnal activity of *H. auripila*, *P. aprica*, *T. bromius* and *T. glaucopis* was characterized by a one-peak curve which reached a maximum between 11:00 and 13:00 h (GMT + 1). During this period, 40–50% of tabanids were collected.

Discussion

Efficiency of traps and daily flight activity

Our results showed that Nzi traps were more effective than Vavoua traps for sampling Tabanidae species. The efficiency of these traps has been previously demonstrated in Africa, North America and Australia (Mihok, 2002; Mihok *et al.*, 2006;
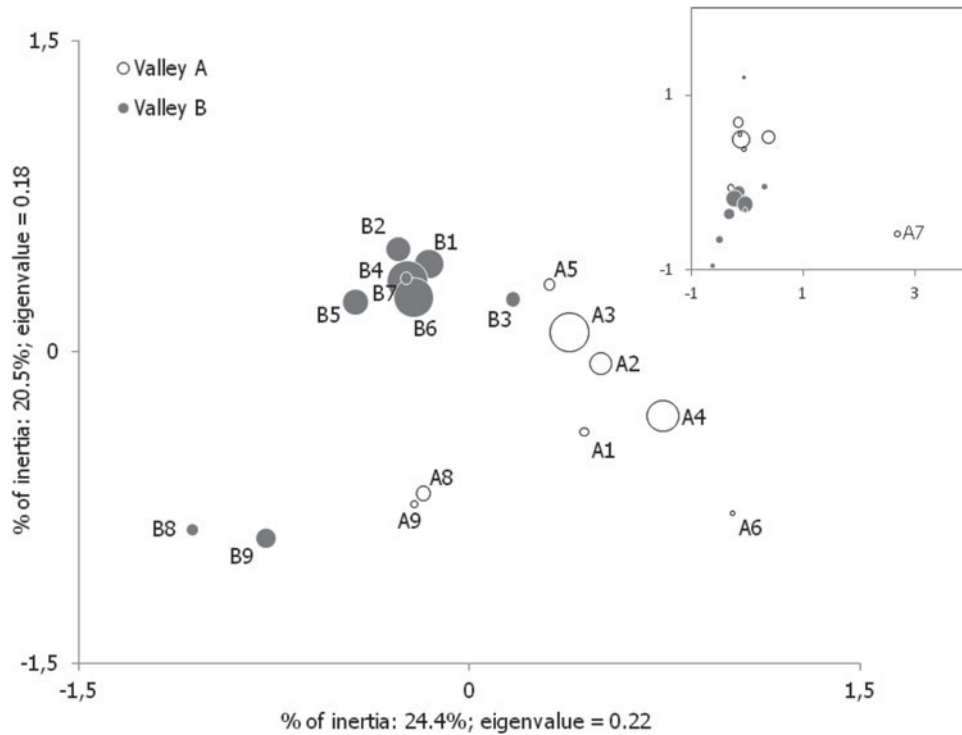
Fig. 4. Plot of trapping locations (without A7) on axes 1–2 of the correspondence analysis (the size of each circle is proportional to the total abundance of catches at each location). The plot of trapping locations including A7 is represented in the inset.
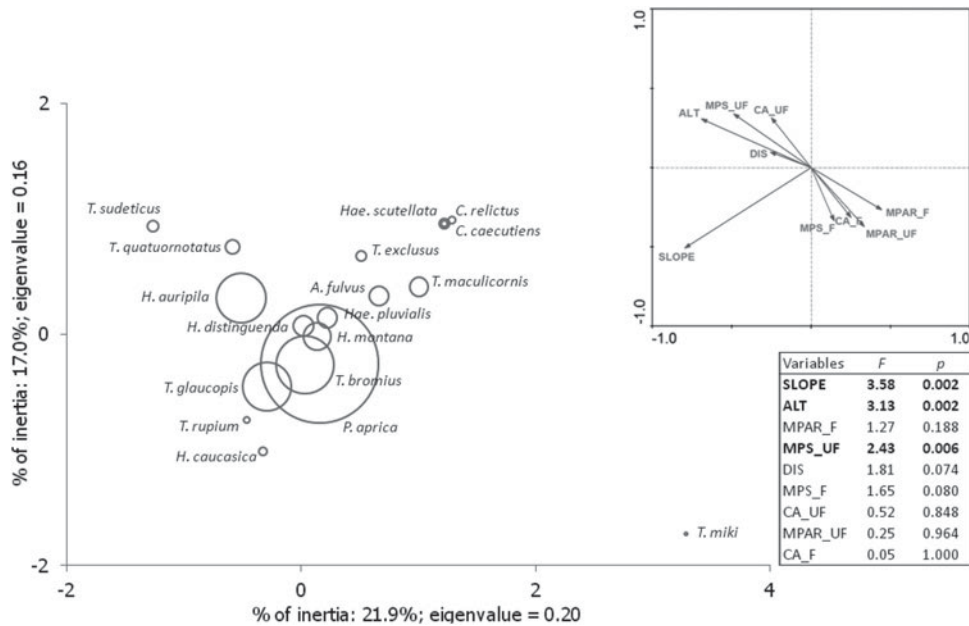

Fig. 5. CCA biplot of the species and the environmental variables (inset). The size of each circle is proportional to the total abundance of catches of each species; complete names in table 2. The environmental variables are the slope of the valley (SLOPE), the altitude (ALT), the distance from the river (DIS), the class area (CA), the mean patch size (MPS) and the mean perimeter area ratio (MPAR) for forested (F) and unforested (UF) patches. The conditional effect (F and P values) after Monte Carlo permutation is reported for each variable. Significant levels are in bold ($P<0.01$).

Table 3. Landscape parameters with significant positive or negative effects on species richness and abundance of tabanids using Spearman's rank-order correlation ($P \leq 0.05$, Monte Carlo permutation test based on 9999 random replicates)

Species richness/species	Parameters (Class)	Spearman r_s	P-value	Valley
Number of species	CA (unforested)	−0.55	0.020	A,B
Number of species	MPS (forested)	0.52	0.028	A,B
Number of species	MPS (unforested)	−0.52	0.027	A,B
Number of species	MPAR (unforested)	0.48	0.041	A,B
P. aprica	Altitude	−0.51	0.029	A,B
P. aprica	MPS (unforested)	−0.52	0.028	A,B
P. aprica	MPAR (unforested)	0.52	0.026	A,B
T. bromius	Altitude	−0.55	0.018	A,B
T. bromius	MPS (unforested)	−0.69	0.044	B
T. bromius	MPAR (unforested)	0.71	0.038	B
H. auripila	Altitude	0.77	0.020	B
T. glaucopis	MPS (unforested)	−0.67	0.050	B

Van Hennekeler et al., 2008). In studies carried out in southeastern France, Nzi traps captured a large number of tabanids in the plain (Baldacchino et al., 2012). Nzi traps seem to be particularly attractive to flies as opposed to other insects, as in addition to horseflies, *Haematobosca stimulans* (Meigen 1824) (Diptera: Muscidae) and other muscid flies that annoy livestock, were also caught (Baldacchino, unpublished data).

Given the high efficiency of the Nzi traps, they were used to estimate the flying activity of tabanids during the day. In the eastern Pyrenees, the four main species, *P. aprica, T. bromius, T. glaucopis* and *H. auripila*, were all active from 9:00 to 19:00 h, with a marked peak between 11:00 and 13:00 h (GMT + 1). In studies carried out in southern Bulgaria, the major peak of activity for *T. bromius* was recorded between 12:00 and 15:00 h (Ganeva, 1999), while in the eastern Caucasus (about 1000 m a.s.l.), *Tabanus* sp. and *Philipomyia* sp. had a conspicuous peak between 16:00 and 18:00 h (Chvála, 1979). Chvála suggested that temperature, relative humidity and thermal winds in the mountains were decisive factors influencing the late afternoon peak of tabanid activity, yet our findings contrasted with these observations. Since meteorological conditions influence the flying activity of tabanids (Van Hennekeler et al., 2011), one might hypothesize that cold temperatures induced by high elevations (our study ranged from 1300 to 2300 m a.s.l.) restricted the favourable period for flight. Our findings revealed that the four main species had a similar pattern of daily activity with a peak at midday, but other tabanid species are known to be active in early or late afternoon, or early in the evening (Chvála, 1979; McElligott & Galloway, 1991; Oliveira et al., 2007). Furthermore, some species have a crepuscular activity, such as *Tabanus equalis* Hine 1923 in Oklahoma (Hollander & Wright, 1980) or *Tabanus paradoxus* Jaennicke 1866 in Europe (Chvála et al., 1972), or exhibit a bimodal pattern of activity, such as *Haematopota* sp. in Uganda (Harley, 1965).

Influence of elevation on species richness and abundance

Insects vary in their population response to altitude (Hodkinson, 2005), yet the influence of elevation on tabanids has been poorly studied. In Tennessee, studies have shown that many species are prevalent only at higher altitudes (600 m a.s.l.), while most species common at lower altitudes (250 m a.s.l.) were also fairly common in upland locations

(Mullens & Gerhardt, 1980). In Croatia, research has found that tabanid abundance and species richness are partially determined by altitude or vegetation along a transect rising from sea level to 1157 m (Hackenberger et al., 2009). In Armenia and the mountainous Nakhitchevan region (1400–1700 m a.s.l.), the flight activity of *Tabanus infestus* Bogatchev & Samedov 1949 has also been found to depend on altitude (Dolin & Andreeva, 1983).

In our study, the CCA showed that altitude had a significant influence on tabanids distribution, as well as a significant effect on the abundance of three out of four main species (positively for *H. auripila*, negatively for *P. aprica* and *T. bromius*), but had no significant effect on species richness. These findings were congruent with published data. *H. auripila*, observed from Scandinavia to Spain, occurs at low altitude in northern latitudes, but in southern Europe is restricted to mountains. On the other hand, *P. aprica* is a typical species of hilly regions, occurring from Spain to Belgium, while *T. bromius* is a ubiquitous species widely distributed throughout the western Palaearctic from Scandinavia to North Africa (Chvála et al., 1972). All these species have previously been observed up to 2000 m a.s.l. in European mountains (Leclercq, 1977).

Slope orientation and seasonal dynamics

The mix of species was different in the two valleys. The CCA showed that community composition of tabanids species was significantly influenced by slope. In the south-facing valley (A), species richness was high, but *P. aprica* largely dominated (64% of catches). Conversely, the evenness was high among the 17 remaining species (Shannon evenness = 0.82). Some typical species were observed close to the lake, such as *Chrysops* and *Haematopota* species, which are known to occur in this type of biotope and do not fly far from the places where the larvae live (Chvála et al., 1972). This explains why trapping location A7 was an outlier with respect to community composition.

The north-facing valley was characterized both by lower diversity and by a lesser degree of domination of *P. aprica* (51%), which was associated with three other abundant species (*T. bromius, T. glaucopis* and *H. auripila*). Together, these four species represented more than 93% of the catches. Such a contrast in fauna related to exposure to sunlight has
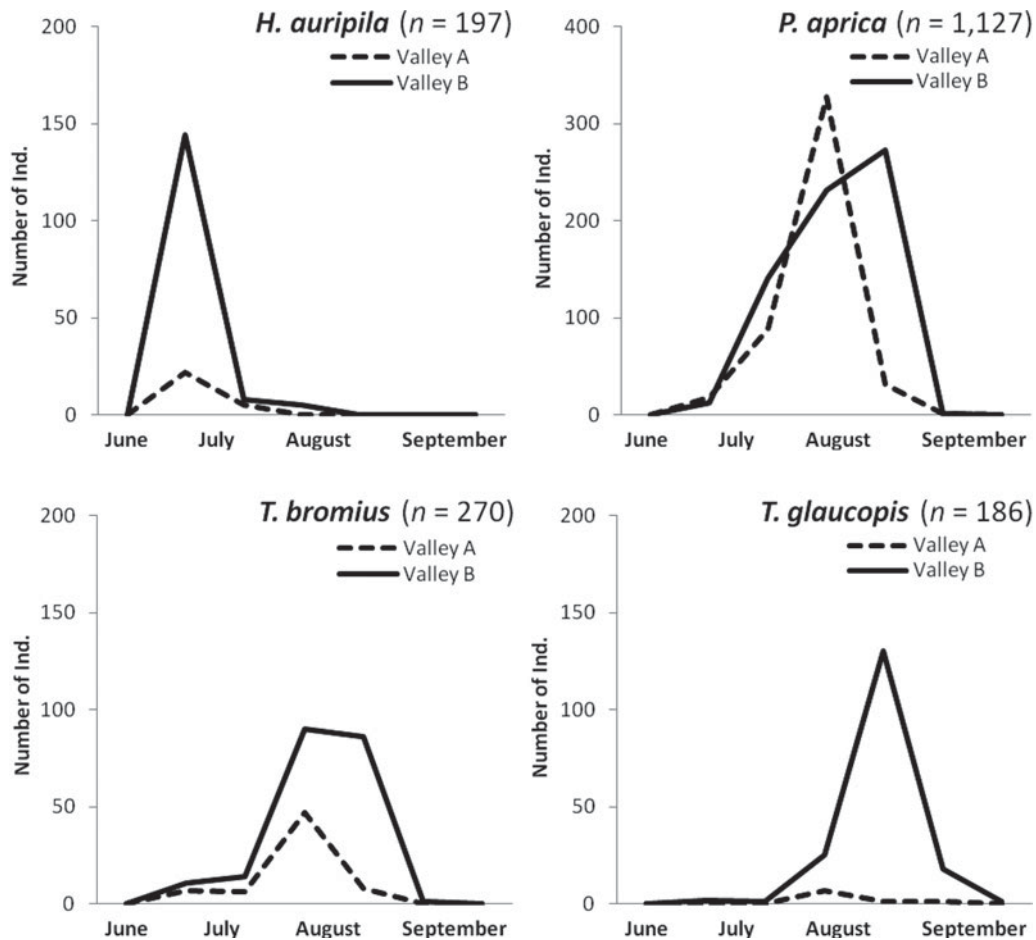
Fig. 6. Catches of the four main species from June to September 2011 in valleys A and B.

been previously shown at lower elevations in the eastern Mediterranean basin, in a study carried out in the Velika Kapela mountains in Croatia (Hackenberger *et al.*, 2009).

A seasonal succession of the main species was observed and may be associated with a strategy to reduce interspecific competition (Barros, 2001). *H. auripila* was active very early in the summer, *T. bromius* and *P. aprica* were active between late July and early August and *T. glaucopis* was active starting from early August. The propitious period for tabanid activity was shorter in the south-facing valley, where 66% of insects were caught during the fifth trapping session (30 July). Conversely, the apparent abundance of tabanids in the north-facing valley (ratio of total catches in valley B/valley A = 1.8) can be explained by the length of the period of activity.

The seasonal dynamics of the most abundant species were consistent with previous studies. In North America, *Hybomitra* species were most active from late May to early July (Mullens & Gerhardt, 1980; McElligott & Lewis, 1998). In the Czech Republic, *H. auripila* emerged first, in June, whereas *T. bromius* emerged in July (Dusbabek, 1986). In Turkey, *Hybomitra* sp. were also the first species to emerge, in late May, whereas *T. bromius* and *P. aprica* peaked in July, with *T. glaucopis* emerging in late July and reaching its peak in mid-August (Kilic, 1993; Altunsoy & Kilic, 2012). In Croatia, *T. bromius* was most active in mid-July or early August, depending on

whether the location was pasture or forest (Krčmar, 2005). In 2007, Hackenberger *et al.* (2009) observed even earlier activity of *T. bromius* in the Velika Kapela mountains (peak of activity in June) and suggested the explanation might have been that the temperature was 3°C above the seasonal average. Hence, seasonal meteorological variability may have a significant influence on the duration of tabanid flight activity, on when they emerge and on their peak of abundance (Krčmar, 2005).

Influence of water bodies and landscape structure

Our study indicated that distance from water (in this case, a river) did not significantly influence the community composition of tabanids. For most tabanid species, the ecological requirements of larvae are poorly known. Wet hollows or somewhat drier edaphic conditions should be suitable laying places for some species, and one might expect that the 19 species observed during the study belong to different ecological groups (Chvála *et al.*, 1972; Andreyeva, 1982; Andreeva *et al.*, 2009). Some species, such as *P. aprica* or *T. glaucopis*, do not depend on aquatic biota for larval development, however, others, such as *T. bromius*, are semi-hydrophilous at larval stage and lay their eggs in slow-moving water bodies (Chvála *et al.*, 1972). Similarly, the presence of *H. auripila* adults near biota such as peat bogs and marshes
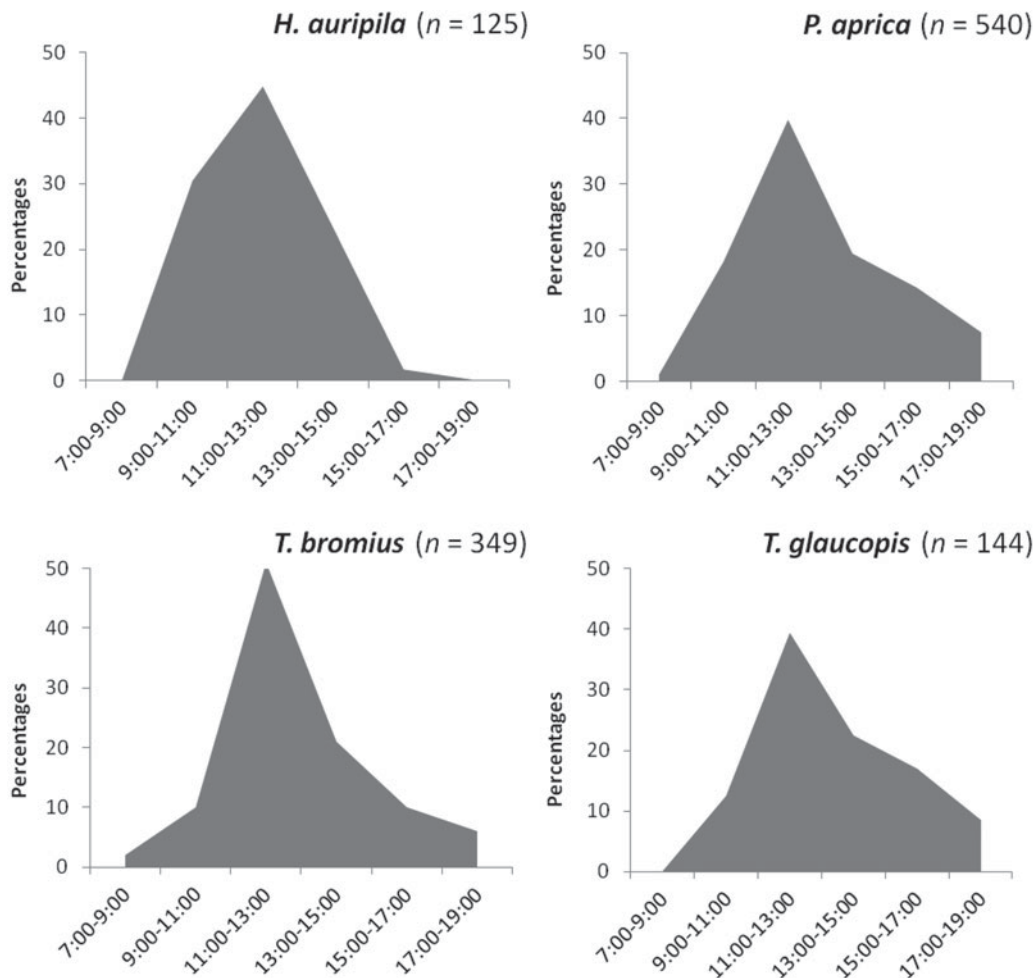
Fig. 7. Daily activity of the four main species between 7:00 and 19:00 h (GMT + 1). The figures show the percentages of the total number of specimens collected during six consecutive 2-hour periods.

suggest that the larvae may be hydrophilous (Chvála *et al.*, 1972). However, given their strong flying ability, (semi-) hydrophilous species are seemingly able to disperse widely, and adults can be collected far from breeding sites.

Although our study did not reveal any significant effect of the presence of a river on the prevalence of tabanids, the community composition was found to be significantly influenced by the distribution of forested and unforested patches in the mosaic of habitats (MPS unforested, $P < 0.01$). Specifically, landscape parameters showed that species richness and abundance of *P. aprica*, *T. bromius* and *T. glaucopis* (78% of total catches) decreased in relation to an increase in the size of clearings, and was enhanced by patch-shape complexity. Therefore, pastures dominated by grasslands and shrubs with patches of forested areas appeared to favour tabanids.

Forested areas are preferential resting sites for tabanids (Okiwelu, 1977). In a mark–recapture study in Texas, *Tabanus abactor* Philip 1936 was found in greater numbers in habitats with cover (e.g. dense leaves and branches) than in areas lacking cover (Kingston *et al.*, 1986). In contrast, open areas are more suitable for host-seeking activity, as a target host tends to attract over a wider range in an open habitat than in a forested one (Barros, 2001).

A study in southern Louisiana showed that *Tabanus* sp. had the greatest host-seeking activity near the edge of a wood, and that this activity decreased significantly at over 200 m from the edge of the wood or more than 20 m into the wood (Sheppard & Wilson, 1977). This preference for pasture–forest ecotone habitats may explain the higher abundance of tabanids trapped in forest clearings than in primary forest in a study in the Amazon (Ferreira-Keppler *et al.*, 2010), and that, in an Ivory Coast study, the largest numbers of tabanids were caught in gallery forests, and the fewest in primary forest (Acapovi, 2001).

Conclusion

The size and physical arrangement of habitat patches in landscapes play a fundamental role in determining the abundance and diversity of insect fauna (Hunter, 2002). Moreover, the complexity of the border between patches at any scale may be as important as the content of the patch, from 'a fly's point of view' (Haslett, 2001). Our study demonstrates

that tabanid distribution was influenced by altitude and forest cover, and particularly by the size and shape complexity of the unforested patches. Our findings indicate that horseflies' preferential habitats are at the edges of their breeding sites, host-seeking areas and resting sites. In the Pyrenees Mountains, the decrease in agro-sylvo-pastoral activities during the last century has favoured the expansion of forest areas and a reduction in open habitats (Gracia *et al.*, 2011). This change may have an influence on the distribution of tabanids in the future, potentially extending their range of activity.

During their period of activity, tabanids reached the maximal peak of abundance in the middle of the summer. Hence, management of grazing areas relative to seasonal occurrence may be an element to consider in integrated management of tabanids (Foil & Hogsette, 1994). Selective grazing in large open areas, well away from wooded habitats and at high elevation levels, may reduce biting activity on livestock during mid-summer. This management could be associated with mechanical control of the population of horseflies using an effective method. Nzi traps have been shown to collect great numbers of tabanids. The setting of such traps close to the pasture–forest ecotone habitats may decrease the populations of tabanids. Such an approach is a potential alternative to the use of insecticides, which are not considered to be very effective against horseflies (Mullen & Durden, 2002), are ecotoxic and have a negative impact on populations of dung-dependent arthropods (Wardhaugh, 2005).

Acknowledgements

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