

# Fine-scale climatic variation drives altitudinal niche partitioning of tabanid flies in a tropical montane cloud forest, Ecuadorian Chocó

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**Abstract.** 1. In montane systems, global warming may lead communities to disassemble by forcing organisms to shift their distributions to higher elevations or by causing the extinction of those that are unable to adapt. To predict which species are most at risk from environmental change, physiological responses to multiple factors must be measured in natural conditions at fine spatial and temporal scales.

2. To examine the potential drivers of elevational distributions in tabanid flies, specimens were exhaustively sampled at three altitudes within a tropical montane cloud forest in Western Ecuador. Observed abundances were then correlated with seven environmental variables measured *in situ*. It was hypothesised that (1) tabanid distributions were significantly associated with particular environmental conditions measured in each altitudinal habitat, and (2) a greater proportion of lowland species were limited to a specific elevation than highland species.

3. Most species occupied well-defined altitudinal niches corresponding to optimal climatic conditions. Colder weather, higher daily temperature variability, and higher levels of moisture seemed to limit most species from establishing in high elevation sites of this mountainous ecosystem.

4. Despite the high dispersal potential of tabanids within the study area, results suggest that most tabanid flies are limited to the subset of altitudes where their climatic requirements are satisfied.

**Key words.** Abundance, altitudinal gradient, Andean slopes, Diptera, elevation, rainfall, Tabanidae, temperature.

## Introduction

Montane systems across the globe, and particularly the Andean cordillera, have been recognised as biodiversity hotspots that harbour rich biotas, often with a high number of endemic species (Orme *et al.*, 2005). In addition, mountains often exhibit regionally unique climatic conditions and are generally less disturbed by human activities than lowlands, often leading them to become a last refuge

for a large number of threatened and endangered species (Killeen *et al.*, 2007; Herzog *et al.*, 2011). Despite the high conservation value of tropical montane ecosystems, precise knowledge about the susceptibility of their biota to climate change is still lacking, though exploratory studies have emphasised the vulnerability of many groups (Pounds *et al.*, 1999; Ponce-Reyes *et al.*, 2012). Due to these attributes, mountain altitudinal gradients provide powerful natural experiments to examine ecological and evolutionary adaptation to local climatic conditions over both short and large spatial distances (Körner, 2007) and may allow for predictions about future range shifts with climate change.

Within invertebrates, the extraordinarily diverse Diptera have been particularly neglected compared to other

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functionally key orders such as Coleoptera or Hymenoptera (Brown, 2005; Donoso *et al.*, 2009). Research on tabanid flies (Diptera: Tabanidae), which are known as important mechanical vectors of pathogen agents to both humans and domestic animals (Krinsky, 1976; Foil, 1989), has focused mostly on taxonomy, local species check-lists, and regional catalogues (Cárdenas *et al.*, 2009). In Ecuador, for example 198 species have been recorded (Cárdenas *et al.*, 2009). While this number represents 16.3% of the Neotropical tabanid fauna, it is still a modest number for Ecuador considering its status as a megadiverse country and many more species are likely to be present. Tabanids are known to occur in a wide diversity of ecosystems from sea level to the highlands up to ca. 4000 m (Cárdenas *et al.*, 2009), but the factors that drive their diversity and distribution patterns along these gradients, including habitat stratification, seasonality and daily activity patterns, are still largely unknown. Studies in other regions (e.g. Chile, Brazil and Hungary) showed that, in the overall, higher temperatures, mist absence, and low rainfall are key drivers of increased adult tabanids abundance (Pino *et al.*, 1973; Ferreira *et al.*, 2002; Herczeg *et al.*, 2015).

It is now well recognised that ectothermal taxa most vulnerable to climate change are concentrated in lowland tropical forests (Huey *et al.*, 2012) where effects are expected to be most severe (Sheldon *et al.*, 2011; Prather *et al.*, 2013). In montane systems warming may force species to shift their distributions from lowlands to highlands, which may cause previously segregated species assemblages to co-occur, a process that has been called community disassembly (Sheldon *et al.*, 2011). In addition, understanding the environmental controls on the distribution of insect disease vectors such as tabanid flies can prove vital when assessing the potential effects of the pathogen dynamics they potentially transmit (e.g. trypanosomes) to the vertebrate wildlife that was previously unaffected. Finally, the ecology and persistence of plants pollinated by tabanid flies may be affected if the ranges of these insects shift with changing climate (Cárdenas *et al.*, 2013). To predict which taxa and interactions will be affected most by environmental change, detailed physiological and behavioural responses to multiple environmental factors must be measured at both large and fine spatio-temporal scales under natural conditions (Willmer, 1982; Helmuth *et al.*, 2010; Huey *et al.*, 2012).

To determine the primary controls on tabanid abundance and distribution, specimens as well as seven environmental variables were sampled every hour from 06.00 to 18.00 hours at three altitudes along a 1-km altitudinal gradient in a tropical montane cloud forest in western Ecuador. Because low variation in ambient temperature in the tropics and in the lowlands may select for organisms with narrow physiological tolerances to temperature, thermal specificity may be higher in tropical mountains compared to those of temperate regions and higher in tropical lowlands than in tropical highlands (Janzen, 1967; Ghalambor *et al.*, 2006). Therefore, it was hypothesised that (i) tabanid

altitudinal ranges will be small and tightly linked to the range of environmental conditions at a given altitude, and (ii) a greater proportion of lowland species will be limited to a single altitudinal area than highland species.

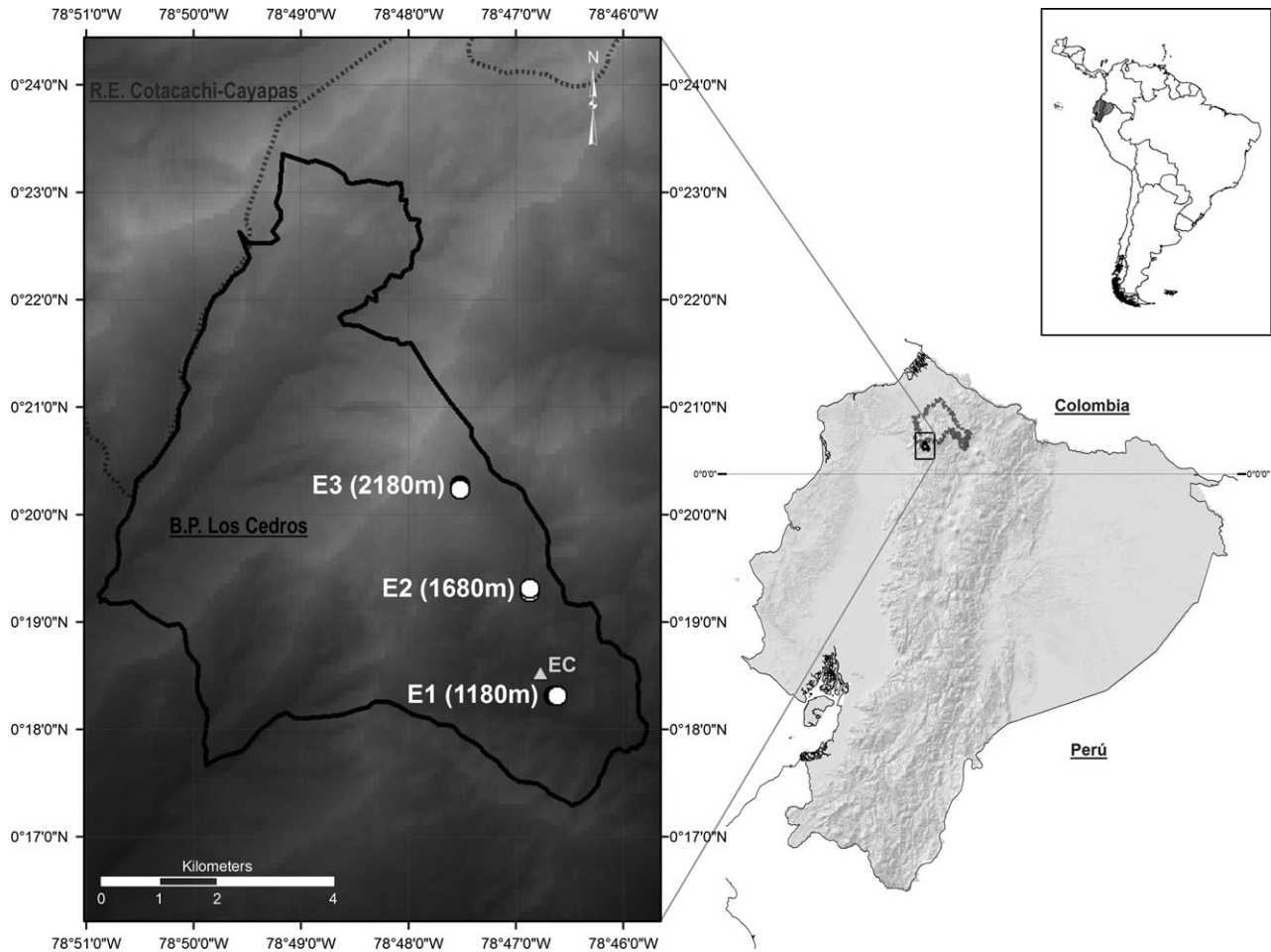
## Materials and methods

### Study area

The study area is located within the Bosque Protector Los Cedros, a 6000 ha Chocóan private tropical montane cloud forest. The protected area spans a strong elevational gradient from 1100 to 2700 m asl, and contains contiguous natural habitat along the entire gradient. Los Cedros is situated on the north-western slopes of the Ecuadorian Andes in the Imbabura Province. The geographical coordinates of the biological station within the reserve are 00°18'31.7"N 78°46'45.9"W and its altitude is 1350 m asl. The reserve is a southern buffer zone for the 243000 ha Cotacachi-Cayapas National Park (Fig. 1), which is surrounded by cattle ranches and farms on other sides. According to Sierra (1999), the study area includes low montane evergreen forest (1300–1800 m asl; canopy: 25–30 m) and montane cloud forest (1800–3000 m asl; canopy: 20–25 m). Its topography has an inclination of 50–70% and average rainfall per year is 2700 mm  $\pm$  536 (Mejía *et al.*, 2005).

### Insect sampling

Tabanids were collected in 2005 during the dry season (July–October; Cárdenas, 2007), the period of the year with the highest tabanid abundance and diversity in humid ecosystems (Buestán, 1980; R. E. Cárdenas, pers. obs.). Three square-designed Malaise traps incorporated with corner baffles (Malaise, 1937; Roberts, 1974) were placed 30 m apart at three separate elevations: E1, 1180 m asl., E2, 1680 m asl. (hereinafter, both considered as lowlands) and E3, 2180 m asl (considered as highland; Fig. 1). As tabanids are attracted to the CO<sub>2</sub> given off by vertebrates, trap bait consisted of 30 kg of dry ice (2 kg per trap, replaced every 24 h for 5 days), which released approximately 10 ml of CO<sub>2</sub>/minute depending on altitude. Trapping efficiency was improved with one human respiration (1.65 m height and 60 kg weight) as an additional CO<sub>2</sub> source (~200 ml of CO<sub>2</sub>/minute; Raymond, 1987; Muirhead-Thomson, 1991), and bicoloured blue and black cotton fabrics as physical attractants (Allan *et al.*, 1987; Sasaki, 2001). Coloured fabrics may have had an additional CO<sub>2</sub> releasing effect of at least 1200 ml of CO<sub>2</sub>/minute per site, based on measurements made by Roberts (1975) with a much smaller black coloured object. In total, at each sampling site there was approximately 1410 ml of CO<sub>2</sub>/minute released (dry ice releasing + human releasing + fabric releasing effect), an optimal amount to attract tabanid flies (see Roberts, 1975).



**Fig. 1.** Geographical location of the three collection sites at Bosque Protector Los Cedros in the north-western Ecuador Andean slopes. E1: 1180 m; E2: 1680 m; E3: 2180 m; EC: Biological Station (1350 m). Los Cedros polygon shapefile downloaded from: <http://reservall.oscedros.org/about/maps/gis-maps/en/>. All other shapefiles and gridfiles at <http://www.diva-gis.org/> and <http://www.worldclim.org/>.

Malaise traps were emptied every hour between 06.00 and 18.30 hours over 20 days, for a total of 250 h of observation per trap per site. Preliminary observations revealed that no tabanid flies were captured in Malaise traps during the night. Specimens were sacrificed using ethyl-acetate, dry collected, pinned *in situ*, and afterwards deposited in the Invertebrates Section of the Museum of Zoology (QCAZ) of the Pontificia Universidad Católica del Ecuador, Quito.

#### *Measurement of environmental variables*

Seven environmental variables were measured every hour at each site while the traps were deployed. Minimum and maximum temperature values (°C) and relative humidity (%) were obtained using a Thermo-Hygrometer (PTH8708 Temperature Humidity Pen, Mannix, New York, NY, USA), which took measurements every 80 seconds and calculated minimum and maximum values from

45 measurements per hour. Atmospheric pressure (Hg) was measured using a digital barometer (Brunton® Sherpa, Atmospheric Data Center, Riverton, WY, USA). Rainfall (mm) was measured using a hand-made pluviometer made of a cylindrical graduated plastic jar (height: 13 cm, diameter: 7.4 cm) with an inverted funnel (diameter: 7.7 cm) glued to the head of the jar to limit evaporation and placed in open areas within the forest. Mist presence/absence was assessed by observation, where >30 minutes of mist presence per hour was considered as presence (1) and, conversely, <30 minutes of mist absence per hour was considered as absence (0). Mist was totally absent at the 1180 m site.

#### *Tabanid fly identification*

Identifications were made by consulting keys and available original descriptions, as well as comparisons with type-specimen illustrations (e.g. Wilkerson, 1979;

Fairchild, 1986; Coscarón & Papavero, 1993) and to identified material from QCAZ Tabanidae collections. Morphological measurements including frontal and divergence indexes of the frons and wing, as well as body size were used for determination. Type-material from tabanid collections of the Natural History Museum of Paris, pictures of type specimens at the California Academy of Sciences (available at <http://research.calacademy.org/research/entomology/typesdb/default.asp>), and the Harvard Museum of Comparative Zoology (<http://insects.oeb.harvard.edu/MCZ/index.htm>) were also accessed for comparisons and helped confirm the identification of some species. A detailed taxonomic discussion of Los Cedros tabanid flies and a full taxonomic bibliography can be consulted in Cárdenas (2007).

#### Data analyses

Mantel tests using Jaccard's presence/absence and Bray–Curtis similarity indexes on log-transformed abundance data were performed to evaluate the independence of the trap replicates at each altitude.

#### Environmental effects on tabanid abundances

A generalised linear model (GLM) was used to test for the effect of five environmental variables (temperature, relative humidity, atmospheric pressure, rainfall and mist) on tabanid abundances at each site. Only mean temperature and relative humidity, instead of maximums and minimums, were considered for this analysis. Mist presence/absence data were first weighed and then averaged. At each altitude, sampling dates were divided into four groups of five consecutive days. Fly abundances were added and environmental data were averaged within each group, as climate may greatly differ from 1 week to another and “zero” presence of one species in a trap did not imply the absence of flying insects in the forest at one particular moment. For this analysis only the seven most abundant tabanid species were considered (i.e. those with  $n$  total  $\geq 20$ ; SIMPER accumulated contribution of ca. 92%; this analysis is explained later, see below). The third day of collection at E2 was not considered for this analysis because an abnormal storm after 16.00 hours greatly increased the rainfall average at this site. Both tabanid abundances and environmental variables were log-transformed before analysis. Model quality was assessed using the corrected Akaike's Information Criterion (AICc) (Hurvich & Tsai, 1989) and contributions of more than two predictor interactions were not taken into account for interpretations. The GLMs were run using R (R Development core team 2013). The sign of the effect of each single variable was evaluated with a multiple linear regression analysis in the Past v. 2.17 software package (Hammer *et al.*, 2001).

#### Altitudinal effect of environmental variables on tabanid assemblages

The relationship between environmental variables at the regional level (including altitude) and tabanid fly species distributions was tested with a partial least squares (PLS) analysis. This analysis combines principal components analysis (PCA) and linear regression, and is particularly useful to predict a set of dependent variables ( $Y$ ) from a set of larger and possibly correlated predictor variables ( $X$ ) when correlations exist between the predictor variables (Balzarini *et al.*, 2008; Carrascal *et al.*, 2009). The PLS was implemented in InfoStat software (Di Rienzo *et al.*, 2012) with species abundances as dependent variables, altitude (collecting site) as a classification variable, and environmental data as predictor variables. This analysis was performed based on total species abundances per altitudinal site and the average of the measured environmental factors for the same period of time. The third day of collection at E2 was also excluded for this analysis due to the extreme weather event. Given that independent variables measured at different scales do not contribute equally to the analysis, they were standardised using Z-score scaling. Standardisation was also applied to the dependent variables to avoid outweigh of highly abundant species. The seven representative species as determined by the SIMPER analysis (see below) were used for the PLS analysis, whereas singletons, doubletons and the remaining species were excluded.

Finally, a PCA was performed on  $\log(x + 1)$  abundance data to examine compositional differences between the three communities, as Hirst and Jackson (2007) found that the PCA method may be most informative when comparing abundance data in an intensively sampled short gradient. A Similarity Percentage (SIMPER) analysis was also performed on log-transformed abundance data for all tabanid taxa to determine which species (variables of the PCA) were most responsible for differentiating sites with different altitudes and the significance of these differences was assessed by an Analysis of Similarities (ANOSIM). These analyses were performed using the Past v. 1.89 software package (Hammer *et al.*, 2001).

## Results

#### Tabanid fly collections

Overall, 5629 individuals from 23 tabanid species belonging to 11 genera and two sub-families (Pangoniinae and Tabaninae) were captured at the three elevations. Of these, five (21.7%) were present at all sites in the gradient (Table 1 in Cárdenas *et al.*, 2013). *Stypommisa* sp. (*pequeñensis* group) was the most abundant species ( $n = 4444$ ), followed by *Tabanus thiemeanus* ( $n = 733$ ), and *Scione* aff. *flavescens* ( $n = 169$ ). These three species represented ca. 95% of the total number of individuals collected. Of all sampled species, 39.1% were singletons and 4.4%



**Table 1.** Species found only at a single collection site. These 14 species represent 60.9% of the total species richness (39.1% at E1; 13% at E2; 8.7% at E3).

E1 (1180 m)	E2 (1680 m)	E3 (2180 m)
<i>Di cladocera</i> <i>argenteomaculata</i>	<i>Esenbeckia</i> aff. <i>nigriventris</i>	<i>Diachlorini</i> sp.
<i>Dichelacera</i> aff. <i>hartmanni</i>	<i>Di cladocera</i> sp. 1	<i>Scione brevibeccus</i>
<i>Di cladocera clara</i>	<i>Scaptia aureopygia</i>	–
<i>Bolbodimyia bicolor</i>	–	–
<i>Di cladocera</i> sp. 2	–	–
<i>Di cladocera</i> sp. 3	–	–
<i>Fidena rhinophora</i>	–	–
<i>Tabanus</i> sp. 2	–	–
<i>Tabanus</i> sp. 3	–	–

doubletons. Twenty-six per cent of the collected flies appear to be new to science and await formal description. Fourteen species representing 60.9% of the total diversity were found exclusively at one of the three sites: nine at 1180 m, three at 1680 m, and two at 2180 m (Table 1). Mantel tests showed that 61% of trap replicates were independent in terms of the composition of collections (see Table S1 for details). Tabanid abundance was higher at mid-elevation, with 3961 specimens collected representing >70% of the total. The high and low altitude sites represented 16.7% and 12.9% of the total abundance respectively.

#### Environmental effects on tabanid abundances

Table 2A shows the results of single environmental factors as drivers of tabanid abundance. At the local scale the GLM analyses showed that temperature alone was significantly and positively associated to tabanid abundance across the elevational range spanned by the study (all  $P < 0.001$ ; all d.f. = 1). Relative humidity showed a similar association with tabanid abundance (all  $P \leq 0.001$ ; all d.f. = 1) except for the 2180 m site, where the association was also positive but not significant ( $P = 0.967$ ; d.f. = 1). Atmospheric pressure showed no significant effect on tabanid abundance (all  $P > 0.05$ ; all d.f. = 1). Rainfall was significantly and negatively associated with tabanid abundance at the 1680 m site only ( $P < 0.001$ ; d.f. = 1), whereas the effect of mist was significant and positive at 1680 m ( $P < 0.001$ ; d.f. = 1) and negative at 2180 m ( $P < 0.001$ ; d.f. = 1). Regarding interacting variables (shown in Table 2B), it was found that the interactions 'relative humidity  $\times$  atmospheric pressure' ( $P < 0.02$ ; d.f. = 1), 'relative humidity  $\times$  mist' ( $P = 0.005$ ; d.f. = 1), 'atmospheric pressure  $\times$  rainfall' ( $P < 0.001$ ; d.f. = 1), 'atmospheric pressure  $\times$  mist' ( $P < 0.001$ ; d.f. = 1), and 'rainfall  $\times$  mist' ( $P = 0.004$ ; d.f. = 1) predicted the tabanid abundance at the 1680 m site.

The PCA ordering method (Fig. 2) significantly discriminated the composition of the three communities.

Components one and two explained 81.4% of the variance, with eigenvalues of 0.795 and 0.492 respectively ( $>0.048$  Jolliffe cut-off significance value). A high rate of species turnover among the collecting sites was also found ( $\beta W = 0.865$ ). ANOSIM based on Euclidean distances revealed significant dissimilarities between groups ( $R = 0.691$ ;  $P < 0.0001$ ). The SIMPER method assessed using seven species explained ca. 92% of the community differentiation: *Stypommisa* sp. (*pequeniensis* group), *Tabanus thiemeanus*, *Scione* aff. *flavescens*, *Stypommisa lerida*, *Dasychela ocellus*, *Tabanus* sp.1., and *Esenbeckia testaceiventris*.

At the regional scale, the first two PLS factors together accounted for a 100% of the relation between tabanid species incidence and environmental factors (Fig. 3). Four groups of flies were distinguished. The first group represented by *T. thiemeanus*, a predominant species at the 1180 m collecting site, positively related to temperature and negatively related to humidity. The second group represented by *Tabanus* sp. 1, *E. testaceiventris*, and *S. aff. flavescens*, all highly abundant at 1680 m, had an equally positive association with temperature, atmospheric pressure, and relative humidity while being negatively associated to rainfall. The third group represented by *S. lerida* and *Stypommisa* sp. (*pequeniensis* group), both highly abundant at 1680 m, was positively associated to a particular range of values of atmospheric pressure and relative humidity. The fourth group represented by *D. ocellus*, a species frequently found at 2180 m, was positively related to rainfall, humidity, and atmospheric pressure and negatively related to temperature.

The 1180 m site had the higher values and lower variation in maximum and minimum temperatures (total average and the difference between maximum and minimum values: 20.3 °C and 0.67 °C, Fig. 4a), and the lower values and higher variability in minimum and maximum relative humidity (86.69% and 4.13%, Fig. 4b). The 1680 m site had medium values and medium variation in maximum and minimum temperatures and humidity (17.35 °C and 0.79 °C, Fig. 4a; 96.85%  $\pm$  2.62 %, Fig. 4b). Finally, the 2180 m site had the lower values and higher variation in maximum and minimum temperatures (15.12 °C and 1.46 °C, Fig. 4a), and the higher values and lower variability in minimum and maximum relative humidity (99.72% and 0.35%, Fig. 4b).

## Discussion

#### Environmental drivers of tabanid abundance dynamics

At the local scale, tabanid flies were more abundant at higher temperatures at the three altitudes. This makes sense as warmer temperatures are often associated with the improvement of adult flight performance in insects (Taylor, 1963; Borror *et al.*, 1976). Insect adult optimal conditions may also be related to temperature and relative humidity fluctuations during larval stages (between

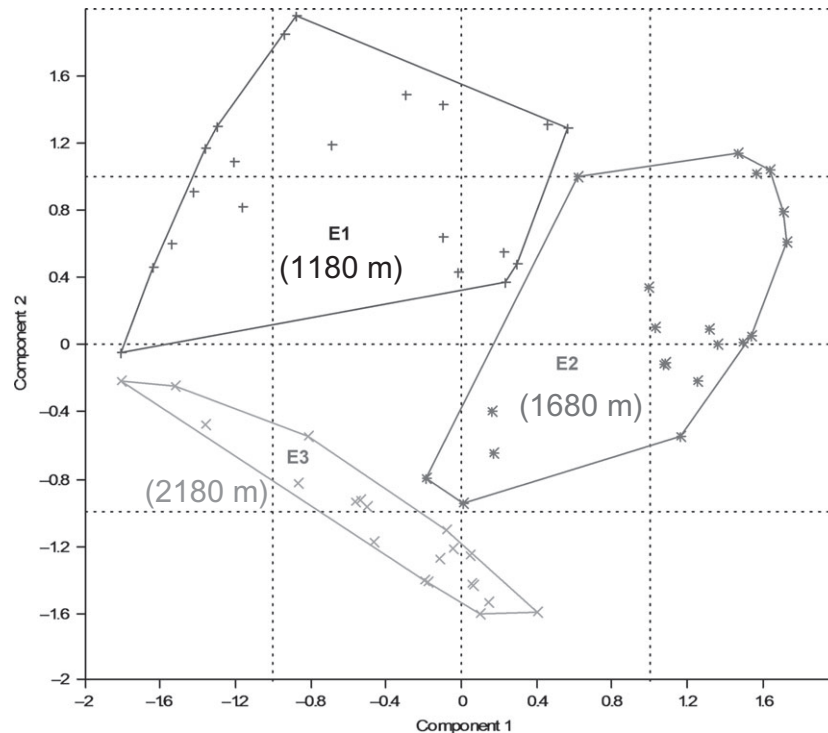
Model	d.f.	Deviance	AICc	F	Sc. dev.	P	MLRA
A – single factors							
E1 (1180 m)							
T	1	11.32	76.86	24.040	21.32	<b>&lt; 0.001</b>	(+)
RH	1	9.94	70.64	15.886	15.09	<b>&lt; 0.001</b>	(+)
PinHg	1	7.26	55.56	0.010	0.01	0.916	(–)
Rainfall	1	7.34	56.08	0.481	0.53	0.465	(–)
E2 (1680 m)							
T	1	49.17	265.91	106.551	82.39	<b>&lt; 0.001</b>	(+)
RH	1	29.90	194.30	10.725	10.78	<b>0.001</b>	(+)
PinHg	1	27.92	184.43	0.873	0.91	0.341	(–)
Rainfall	1	32.49	206.26	23.604	22.74	<b>&lt; 0.001</b>	(–)
Mist	1	30.56	197.45	14.018	13.93	<b>&lt; 0.001</b>	(+)
E3 (2180 m)							
T	1	12.60	84.01	186.930	81.40	<b>&lt; 0.001</b>	(+)
RH	1	2.31	2.62	0.002	<0.01	0.967	(+)
PinHg	1	2.31	2.62	<0.001	<0.01	0.991	(+)
Rainfall	1	2.32	2.70	0.071	0.08	0.780	(–)
Mist	1	3.88	27.50	28.523	24.88	<b>&lt; 0.001</b>	(–)
B – interactions							
E1 (1180 m)							
None	–	–	–	–	–	–	–
E2 (1680 m)							
RH × PinHg	1	13.31	97.7	5.106	5.63	<b>0.018</b>	–
RH × Mist	1	13.52	99.96	7.207	7.89	<b>0.005</b>	–
PinHg × Rainfall	1	15.21	116.96	24.151	24.89	<b>&lt; 0.001</b>	–
PinHg × Mist	1	15.26	117.38	24.595	25.31	<b>&lt; 0.001</b>	–
Rainfall × Mist	1	13.54	100.21	7.443	8.14	<b>0.004</b>	–
E3 (2180 m)							
None	–	–	–	–	–	–	–

AICc is the corrected Akaike's Information Criterion for the initial model after removal of the "effect" term. *P*-values test the hypothesis that the suppression of the 'effect' term provides no better fit than the initial model. Mist was not analysed in E1 because it never occurred in this part of the forest. For interacting variables, only significant results are shown when they exist. Sc. dev., scaled deviance; MLRA, Multiple linear regression analysis; T, temperature; RH, relative humidity; PinHg, atmospheric pressure; (+), positive relationship; (–), negative relationship.

maximums and minimums) given that larvae rearing temperatures in Diptera have shown to be strongly related to flight performance as well, where low rearing temperatures may implicate better cold-flight performance (Frazier *et al.*, 2008). With respect to mist-related factors (i.e. lower temperatures, higher relative humidity, and frequent rainfall events), results of this study were not consistent with a previously observed negative effect on tabanid flight activity (Pino *et al.*, 1973; Oliveira *et al.*, 2007). Intriguingly, while relative humidity showed a significant positive effect on tabanid abundance at the 1180 and 1680 m sites, and rainfall had a significant negative effect at the 1680 m site, mist showed both positive (1680 m) and negative (2180 m) effects at different elevations. It is known that insects have the ability to finely exploit graded habitats, escaping from harsh ambient conditions into more favourable micro-niches (Willmer, 1982). This capability to regulate daily activities is a critical behavioural mechanism determining body temperature in terrestrial ectotherms (Stevenson, 1985). In accordance with

this, Cárdenas *et al.* (2013) found large differences in flight activity of *Stypommisa* sp. (*pequeniensis* group) along this same altitudinal gradient. Based on morphometric analyses, the authors suggested that this tabanid fly species would be able to migrate uphill or downhill in the forest to avoid harsh weather conditions. The authors proposed that some tabanid species may be capable of shifting from one environmentally suitable place to another – within the forest and at defined hours of the day – while seeking nutritional resources (i.e. mammals' blood and/or flowers' nectar; generally in a ≤500 m altitudinal range). Seeking warmer microhabitats in the forest may thus be a common behaviour in insects, as a way to warm wings to regulate body temperature and prepare for flight (Wasserthal, 1975; May, 1979). These behavioural patterns could explain the results observed in this study in three ways. First, during heavy mist events at 2180 m, for example some tabanid species may fly downhill to where weather conditions are less harsh, yet could remain active in spite of the mist presence at lower elevations as they

**Table 2.** Results of GLM analysis for five environmental factors on seven tabanid species abundances. Bold *P*-values highlight statistical significance.



**Fig. 2.** Principal Components Analysis discriminating the three tabanid fly communities along the elevational gradient at Bosque Protector Los Cedros. E1 = 1180 m (black polygon and cross symbols); E2 = 1680 m (dark grey polygon and asterisks); E3 = 2180 m (grey polygon and crossmarks). Component 1: % variance = 50.279; eigenvalue = 0.795; component 2: %variance = 31.127; eigenvalue = 0.492; Jolliffe cut-off = 0.048 (< Component 4 eigenvalue which is of 0.061).  $\beta W = 0.865$ . Seven species explained the ca. 92% (cumulated contribution) of the communities' differentiation (SIMPER analysis): *Stypommisa* sp. *pequeniensis* group; *Tabanus thiemeanus*; *Scione* aff. *flavescens*; *Stypommisa lerida*; *Dasychela ocellus*; *Tabanus* sp.1; *Esenbeckia testaceiventris*.

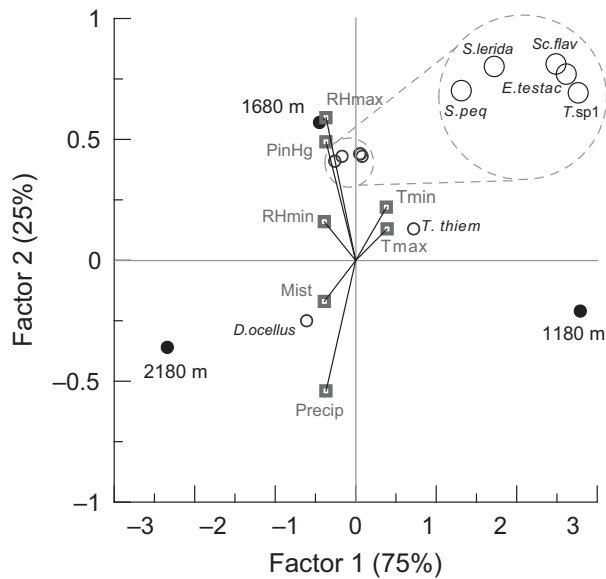
would be naturally adapted to lowlands (Ghalambor *et al.*, 2006) probably because of warmer temperatures. Second, the significant effects of several moisture-related interacting variables at the 1680 m site may be explained by tabanids within-forest movements. In the search of suitable microclimatic environments, flies must have flown to and through this site at diverse weather conditions moments, so that variables might have affected complementarily the tabanid abundances in this site. Finally, contrary to the literature findings (e.g. Pino *et al.*, 1973; Oliveira *et al.*, 2007), the positive effect of relative humidity on tabanid abundance along the gradient may be due to the fact that organisms in the study area are adapted to the humid conditions characteristic of tropical montane cloud forests (minimum average at 1180 m: 84.8%; maximum average at 2180 m: 99.9%).

#### *Environmental factors limiting tabanid regional distribution*

At the regional scale, this study showed that the distribution of Tabanidae along the altitudinal gradient may be limited by particular combinations of climatic conditions. Although the lowest site was most diverse, PLS results showed that most species seemed to find optimal habitat

at the 1680 m site, where they were positively associated with relative humidity, atmospheric pressure, and temperature, but negatively associated to rainfall. Both the 1180 and 1680 m sites had relatively higher temperature averages and lower humidity values, rainfall accumulation, and mist events compared to the 2180 m site. The 1180 m and 1680 m sites also had lower variation in minimum and maximum temperatures compared to the 2180 m site, and higher variability in minimum and maximum relative humidity values (note curves in Fig. 4 had very little overlap). This suggests that the higher temperatures and fluctuating relative humidity (that could reach relatively dry moments during the day) in the mid and lower elevations may represent optimal developmental conditions for most of the study species. Hence, the area between the 1680 m and the 2180 m sites could be considered a climatic and physiological barrier for the optimal development and dispersal of most of the species in this forest, which would be in accordance with Janzen's (1967) hypothesis on climatic tolerances in tropical mountains.

The fact that some abundant tabanid species were mainly found at one particular altitude may indicate strong and well-established habitat preferences. In this survey, for example 92% of *D. ocellus* specimens were collected at the 2180 m site, whereas it was very rare at



**Fig. 3.** Partial least square (PLS) ordination diagram showing the effect of altitude (1180, 1680 and 2180 m; filled circles), relative humidity (RH, maximum and minimum), rainfall (Precip), atmosphere pressure (PinHg), temperature (T, maximum and minimum) and mist environmental variables (empty squares) on seven tabanid fly species abundances (empty circles) at Bosque Protector Los Cedros. *D. ocellus*, *Dasychela ocellus*; *E. testac*, *Esenbeckia testaceiventris*; *Sc. flav*, *Scione aff. flavescens*; *S. lerida*, *Stypommisa lerida*; *S. peq*, *Stypommisa sp. pequeniensis* group; *T. sp1*, *Tabanus sp. 1*; *T. thiem*, *Tabanus thiemeanus*.

1680 m (8%) and completely absent at 1180 m. Likewise, 85% of *E. testaceiventris*, 82% of *S. aff. flavescens*, 76% of *Stypommisa sp. (pequeniensis group)*, 73% of *S. lerida* and 72% of *Tabanus sp.1* specimens were collected at the 1680 m site. Accordingly, the PCA ordering method iden-

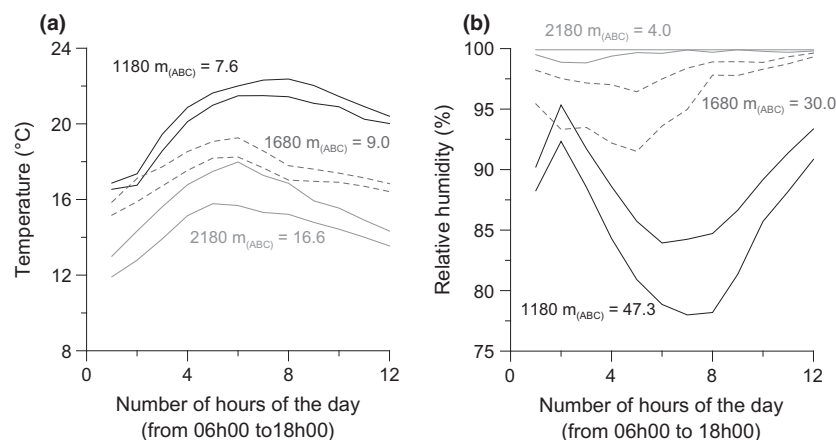
tified three different assemblages along the gradient corresponding to each of the collecting sites, and the PLS analysis showed that species were associated with particular climatic factors. These results strongly suggest that tabanid assemblages are mostly limited to specific elevations where optimal physiological environments exist.

## Conclusion

Supporting the first hypothesis, the results suggest that tabanid flies tend to confine daily activities to physiologically optimal habitats. Even if potentially capable of migrating in search of resources or avoiding harsh weather conditions, highland sites with colder and moister averages and large diurnal temperature variation seem to exclude most species from establishing. This may explain in part why most common lowland species, when compared with those in the highlands, are more restricted to the lowland sites, which is in agreement with the second hypothesis. Whether the altitudinal distribution of tabanid flies in the Andes follow the Rapoport's rule (Stevens, 1992), where highland species have broader habitat ranges compared to lowland species due to higher physiological tolerances to harsh environmental conditions, is yet to be tested.

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**Fig. 4.** Average variation in the minimum and maximum temperature (a) and relative humidity (b) at the three altitudinal sites for 12 hours a day. The 'area between the curves' (ABC) for each site are given (1180 m = solid black lines; 1680 m = dashed dark grey lines; 2180 m = solid grey lines). ABCs values were calculated using LoggerPro v. 3.6 (Vernier Software & Technology, Beaverton, OR, USA).



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### Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: doi: 10.1111/icad.12146:

**Table S1.** Mantel tests showing the correlation of replicate-traps tabanid collections along the altitudinal gradient at Los Cedros Tropical Mountain Cloud Forest (Ecuador) using Bray–Curtis and Jaccard's indexes of similarity.

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