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Testing the Species pool hypothesis for mire vegetation: exploring the influence of pH specialists and habitat history

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The Evolutionary species pool hypothesis (ESPH) predicts that historically more common habitats will be richer in species because they have had greater opportunity for the evolution of suitably adapted species. We explored the relationship between mire species richness and pH, an important environmental variable in mires, in two regions that differ in habitat pH distribution: the West Carpathians and Bulgaria. Mire habitats in both the West Carpathians and Bulgaria demonstrate support for the ESPH prediction that habitats with more common pH values host more species. We also explored the influence of habitat history by examining the distribution of generalists and specialists along gradients of habitat pH, using extensive community-level vegetation data from European mires in these two regions. We found a striking pattern with the distribution of pH-specialists having three distinct peaks in both regions, whereas the total species pool peaked in near neutral pH habitats in both regions. Because the peaks in specialist richness do not correspond to regional pH distribution patterns, we hypothesize that historical explanations may be important, and that habitats currently rich in pH-specialists may have historically acted as pleniglacial refugia for many mire species. Our findings support the general predictions of the ESPH, but further suggest that historical processes such as patterns of glacial refugia, may significantly influence contemporary species distributions and the diversity of plant species in mire habitats.

It has recently been demonstrated that species richness patterns can be explained, in part, by the evolutionary species pool concept which predicts that habitats that have been historically more common have evolved a larger pool of adapted species and therefore are both regionally and locally richer in species. This concept has been used to explain the unimodal pattern of plant species richness along productivity gradients (Taylor et al. 1990, Aarssen and Schamp 2002, Schamp et al. 2002) and to interpret patterns of terrestrial plant and diatom species richness along pH gradients (Pärtel 2002, Pither and Aarssen 2005a).

Many studies have tested the Evolutionary species pool hypothesis (ESPH) by correlating contemporary habitat commonness and species pool size (Schamp et al. 2002, 2003, Pither and Aarssen 2005a), assuming that the present-day habitat commonness approximates historical patterns of commonness. The results of Ewald (2003), however, show that contemporary distributions of habitat commonness may poorly represent historical patterns in some regions that have been greatly affected by temporal fluctuations in environmental conditions. For example, Ewald (2003) argues that contemporarily common acidic soils are characterized by a relatively small pool of acidophytes in Europe that result from acidophyte extinctions during the Pleistocene, when base-rich environments predominated.

There are several other constraints to the wider application of the evolutionary species pool concept: (1) the correlation between local species richness and species pool size does not necessarily imply that the former is determined by the latter (Herben 2000, Wilson and Anderson 2001); (2) a larger species pool size may result also from higher beta-diversity, i.e. habitat heterogeneity (Pither and Aarssen 2005a); (3) the evolutionary species pool concept refers to the landscape as a whole, but the species richness pattern in particular habitat types may be influenced by vicinism/ mass effects (Zonneveld 1995); (4) particular habitat types may differ in the ratio of generalist and specialist species, while the number of specialists may reflect habitat history more closely.

Mires in the West Carpathian and Bulgarian regions present a particularly unique opportunity for testing the ESPH. First, the ESPH has never been tested for mire communities. Secondly, many large-scale studies report a bimodal distribution of water pH across mires, with one mode in relatively acidic habitats ($\sim$pH $=4$) and the second in circumneutral values ($\sim$pH $=7$) (Gorham and Janssens 1992, Vitt 2000, Wheeler and Proctor 2000, Sjörs and Gunnarsson 2002). This bimodal distribution of pH commonness has generally been explained by the existence of two different buffer systems and the chemical instability of the intermediate pH. No study to date has explored the influence of habitat commonness on richness when habitat variables have multimodal distributions. Habitat pH has been identified as a crucial factor determining floristic composition in mires (Vitt 2000, Hájek et al. 2002, Tahvanainen 2004), and is frequently a good predictor of floristic species richness. Frequently, local species richness in mire vegetation has been observed to increase with increasing habitat pH and peak near circumneutral pH levels (Glaser et al. 1990, Vitt et al. 1995, Gunnarsson et al. 2000, Hájková and Hájek 2003, Tahvanainen 2004).

It has been further proposed that the influence of habitat commonness will be more pronounced on species with narrow niches (pH specialists hereafter for convenience). Therefore, we investigate whether the richness of specialists more closely matches the distribution of habitat pH and subsequently whether any relationship between species richness and habitat commonness in mires results from the simple numerical superiority of generalists above specialists that often occurs in nature (Pither and Aarssen 2005b).

Methods

Data collection

We collected the following data: (1) plant species richness, abundance and water pH and conductivity for 676 plots in mires and open springs gathered throughout the West Carpathians (central Europe); (2) similar plant species richness, composition and water chemistry data for 483 plots in similar habitats in Bulgaria (southeast Europe), located ca 800 kilometres from the first region. The latter region is noteworthy because it contains many species that are disjunct from their central range and several species that appear to have wide realized niches in Bulgaria while occupying a more narrow niche in the West Carpathians (Hájková et al. 2007). We surveyed 16 m^2 plots in each target community, which is the standard for such habitats (Chytrý and Otýpková 2003). Target habitats were distributed mostly as small isolated islands in our study areas and the standard sample plots typically covered a large fraction of their extent. Smaller plots were used when vegetation islands in target communities were smaller than 16 m^2 area. Species composition of the vegetation, including both bryophytes and vascular plants, was recorded within all sample plots. Floristic abundance was measured as percent coverage per species per plot, transformed into nine-degree scale according to van der Maarel (1979).

In both regions, water pH and conductivity were measured using a portable pH-meter and conductometer. We used a consistent sampling protocol to minimize sampling biases (Tahvanainen and Tuomaala 2003); measurements were conducted directly in microsites best supplied by water in the central parts of the springs. A small shallow pit was excavated and spring water was allowed fill this pit, and clarify before pH and conductivity measurement. Whenever spatial variation of water pH or conductivity was observed, replications were conducted at different spatial locations within the plot and pH was quantified as the arithmetic mean of these samples. Conductivity caused by H^+ ions was subtracted in acidic waters with pH <5.5 (Sjörs 1952). Corrected conductivity was used as a proxy for total mineral richness of the water sample and correlated most strongly with the sum of calcium and magnesium concentrations (Sjörs and Gunnarsson 2002, Horsák 2006). Seasonal variability could not seriously bias our results as seasonal monitoring conducted in the West Carpathians showed a high temporal stability of pH along the entire pH/calcium gradient within fens (Hájek and Hekera 2004).

Since habitat heterogeneity could seriously bias our results, we calculated Whittaker's beta-diversity index (Koleff et al. 2003) for vegetation plots with the same pH value rounded at 0.1 units. The value of this index was plotted with specialist distributions.

The fact that the vegetation plots were of slightly unequal size could influence the analysis of local species richness. Therefore we removed the area effect by calculating linear regressions of the local species richness on log-transformed plot size. Logarithmic transformation was used to fit the empirical species–area relationship. We then regressed standardized residuals from these relationships against observed pH using the quadratic term (Chytrý et al. 2003), because preliminary analysis using locally weighted linear regression suggested a non-linear relationship (not shown).

Evolutionary species pool hypothesis

We generated frequency histograms for the distribution of pH in each of the two study regions and explored relationships between water pH and local species richness using linear and quadratic regressions to establish the relationship between habitat pH and species richness for each region, and to establish ESPH predictions for the shape of habitat distributions (Schamp et al. 2002). We tested for predicted distribution shapes using the Monte Carlo test outlined in Schamp et al. (2002), as well as a series of chi-square tests for classifying distribution shape. The first step in the chi-square analysis of distribution shape was to divide the pH gradient for each region into equal thirds. We then tested whether the number of sample data points within these sections differed from what would be expected if samples were drawn from a uniform distribution. The second step employed two similar tests but compared the first and second third of the data, and then the second and last third of the data to determine whether the middle third of the gradient contained significantly more data points than either the first or last third (i.e. was hump shaped). This is a second test for a hump-shaped distribution, and simultaneously tested for an increasing-triangular distribution shape.

The role of specialists

To designate species as specialists vs generalists in each region, we created species response curves to single environmental factors following model-type 5 of Huisman et al. (1993) which is based on logistic regression and follows the algorithm of Oksanen and Minchin (2002). Only species with more than 10 occurrences were analysed as it is difficult to demonstrate whether a species is a generalist or specialist for rare species (Pither and Aarssen 2005b). For each species, we determined the response interval (i.e. species tolerance), defined as the distance between parts of the gradient where the predicted probability of occurrence reached more than half of the maximum predicted probability of occurrence (Schröder et al. 2005). We refer to these parts of the gradient as the minimum and maximum of the response interval (Fig. 1). Next, we calculated the number of species whose tolerances overlap with each pH value rounded to 0.1 units (potential species pool according to Pärtel et al. 1996). The same procedure was repeated only for pH-specialists, defined arbitrarily as one third of the species with narrowest response to pH. The relative proportion of all species and relative proportion of specialists were plotted against observed pH values to visualize differences in the proportion of the total pool of species realized along the pH gradient in each region and for comparison between the two data sets. As our definition of specialists is arbitrary, we also created curves based on thresholds of one half and two thirds of the species with the narrowest response to pH. The resulting curves were intermediary and therefore only two curves, one based on the entire data set and the second based on one third of most specialized species are presented.

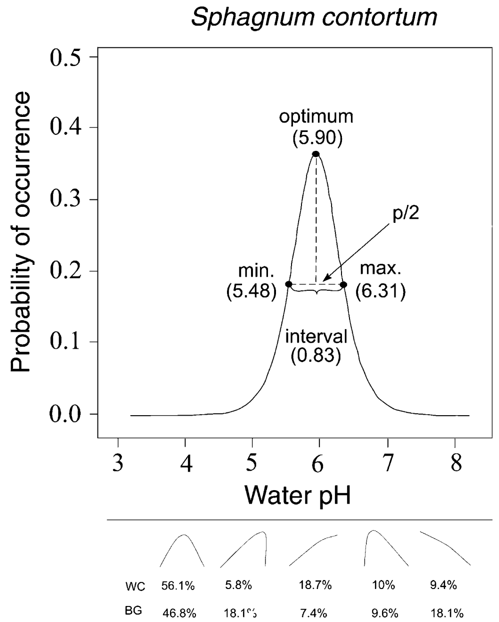

Fig. 1. A graph of the method of determining species optima and tolerances. This example for a single species (*Sphagnum contortum*) presents the response of this species to pH in the West-Carpathian data set. The response interval (species tolerance) was defined as the distance between the parts of the gradient where the predicted probability of occurrence reached more than half of the maximum predicted probability of occurrence. The lower frame shows the frequency (in percentage) of various response types (unimodal, skewed unimodal, monotonic) in both data sets. WC = West Carpathians; BG = Bulgaria.

To remove the potentially confounding effect of casual species (i.e. poorly adapted species present only because of strong mass effects from nearby source populations), we have excluded from our analysis of specialists those species that are widespread across different habitats and whose occurrence in mires can be attributed to vicinism (data sets 1 and 2). This did not alter the results at all and we therefore present figures and tables obtained from reduced data sets to allow a clear interpretation of the results with respect to target habitat type. We also tested the robustness of our results by using two different methods of processing our data sets. In poorly productive, nutrient stressed fens, such as those explored in this study, competition is believed to play a small role (Grime 1979), and we therefore assume that competition will have, at most, a minor impact on realized niches in these communities. This is supported by the fact that our target vegetation is nutrient-limited, often polydominant, with a sparse herb layer. Rydin and Barber (2001) have demonstrated that competitive exclusion is low even in *Sphagnum*-dominated peatlands.

Results

Evolutionary species pool hypothesis

We explored the ESPH prediction that distributions of pH commonness can explain variation in species richness using the criteria set forth by Schamp et al. (2002). There are two steps in this test. First, the relationship between species richness and pH must be established. Second, based on the observed relationships, predictions can be made about the shape of the pH distribution which can then be tested (Schamp et al. 2002). In both the West Carpathian and Bulgarian regions, residual species richness was significantly positively related to habitat pH (West Carpathians: $n = 664$, $m = 0.485$, $r = 0.39$, $p < 0.001$; Bulgaria: $n = 414$, $m = 0.334$, $r = 0.070$, $p < 0.001$). We further tested for a 'hump'-shaped relationship between residual richness and found significant quadratic regressions for both regions (West Carpathians: $r = 0.417$, $p < 0.001$; Bulgaria: $r = 0.10$, $p < 0.001$; Fig. 2). Based on these findings, the ESPH predicts that pH distributions in these regions should be both triangular increasing (i.e. high end of the gradient significantly more common), and 'hump'-shaped (i.e. middle portion of the gradient more common). Taken together, both regions are predicted to have left-skewed distributions of habitat pH. Using the Monte Carlo test of Schamp et al. (2002), as well as a series of chi-squares tests, we confirmed that the distribution of habitat pH in the West Carpathians is indeed triangular increasing (Table 1). We also found support for the ESPH prediction that the pH distribution in the West Carpathians is significantly hump-shaped (Monte Carlo test), but chi-squares tests did not support this finding (Table 1). Both statistical tests support the predicted triangular-increasing, and 'hump'-shaped pH distribution for Bulgarian mires (Table 1). The p-value listed for the chi-square tests require some explanation. Where the p-value is listed as significant at $p < 0.0001$, this signifies that the middle third of the pH distribution contained significantly more data points than both the first third, and last third of the pH gradient (i.e. both tests were significant with $p < 0.0001$). Where the p-value is listed as $p > 0.05$, this signifies that at least one of the two chi-square tests were not significant (in this case it was one of the two that was not significant). The summary statistic for the 'hump'-shaped distribution test in Table 1 represents the overall significance of these two sub-tests.

The role of specialists

Both independent mire data sets show an uneven distribution of specialists along the pH gradient with three distinct peaks, whereas the total species pool peaks in the neutral part of the pH gradient (Fig. 3). The Carpathian region has a high number of specialists in the subneutral pH between 6.0–6.2, whereas acidic (3.5–3.7) and alkaline peaks (7.5–7.7) are less pronounced. In the Bulgarian mire data set, the subneutral peak (5.8–6.0) is less pronounced than acidic (4.6–4.8) and alkaline (7.5–7.7) ones. Altogether 93% of pH-specialists in Bulgaria contributed to one of the three peaks, while 75% of specialists contributed to the peaks in the Carpathians (Appendix 1).

In the Carpathians, the peak in acidic specialists is formed mostly by species found predominantly in ombrotrophic hummocks while both bog and fen species make up this peak in Bulgaria. In both regions, the subneutral peaks are formed by species found predominantly in rich fens and/or subalpine springs and by calcitolerant *Sphagnum* species. For example, *Sphagnum contortum* contributes to this peak in both data sets. The alkaline peaks in both regions are formed by the species of calcareous fens and tufa-forming springs (Appendix 1). Changes in beta-diversity along the pH gradient do not correspond with the shape of the "trimodal" distribution of pH-specialists.

The same pattern in specialist distribution is obtained when logarithmically transformed conductivity values are used instead of pH in both mire data sets (Appendix 2), with a small difference in the Carpathian data set where a small additional peak in salt-rich alkaline fens appears.

The revealed pattern of pH-specialist distribution is apparently not caused by any statistical bias caused by uneven distribution of pH classes in our data sets. The pH distributions for both regions show no evidence

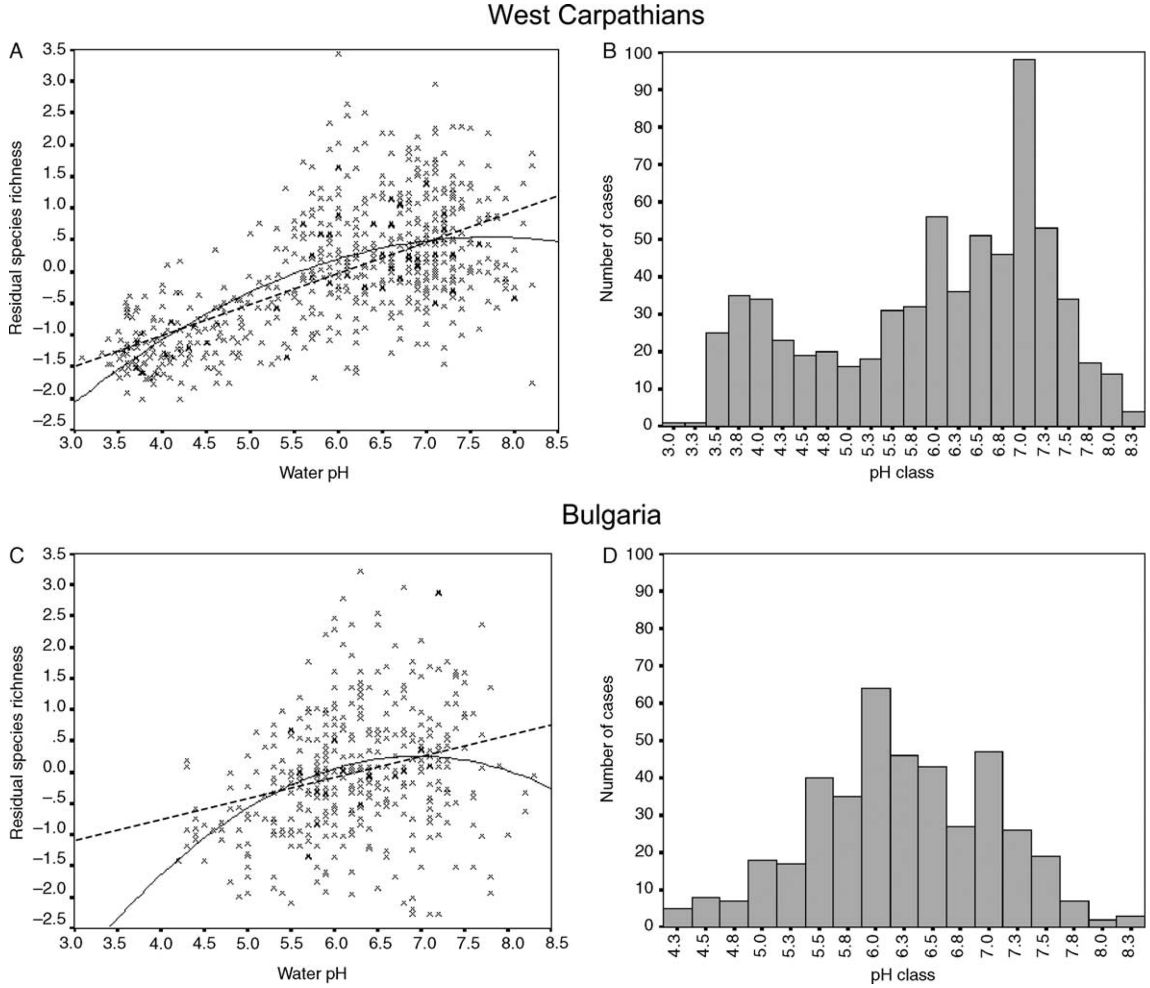

Fig. 2. Graphs depicting the relationships between local species richness and habitat pH and associated frequency distributions of habitat pH for the West Carpathians (panel A–B), and Bulgaria (panel C–D). Standardized residuals from the linear regressions of local species richness on log-transformed size of relevé plots are plotted against measured water pH (panels A, C). Curves are fitted using both linear (dotted line) and quadratic (full line) regression. All regressions were significant (West Carpathians: linear, $r = 0.39$, $F = 418.423$, $p < 0.001$; quadratic, $r = 0.418$, $F = 237.64$, $p < 0.001$; Bulgaria: linear, $r = 0.070$, $F = 31.810$, $p < 0.001$; quadratic, $r = 0.104$, $F = 23.83$, $p < 0.001$).

of trimodality, and peaks in specialist richness do not correspond with the apparent bimodality of the pH distribution in the Carpathian region (categories 3.9–4.1 and 6.9–7.1 in Fig. 3). The distribution of pH values in Bulgaria is left-skewed and does not match the trimodal distribution of specialists.

Table 1. Summary table of species pool hypothesis predictions about pH distributions and the result of subsequent distribution tests. Bold p-values are significant and support the prediction of the evolutionary species pool hypothesis.

Region	pH-richness	pH distribution prediction	Monte Carlo distribution test	Chi-square distribution test
West Carpathians	+ linear	increasing	**p < 0.002**	**p = 0.0001**
	quadratic	hump-shaped	**p = 0.0177**	p > 0.05*
Bulgaria	+ linear	increasing	p = 0.2756	p > 0.05*
	quadratic	hump-shaped	**p = 0.0001**	**p = 0.0001**

'+' designates a significantly positive slope.

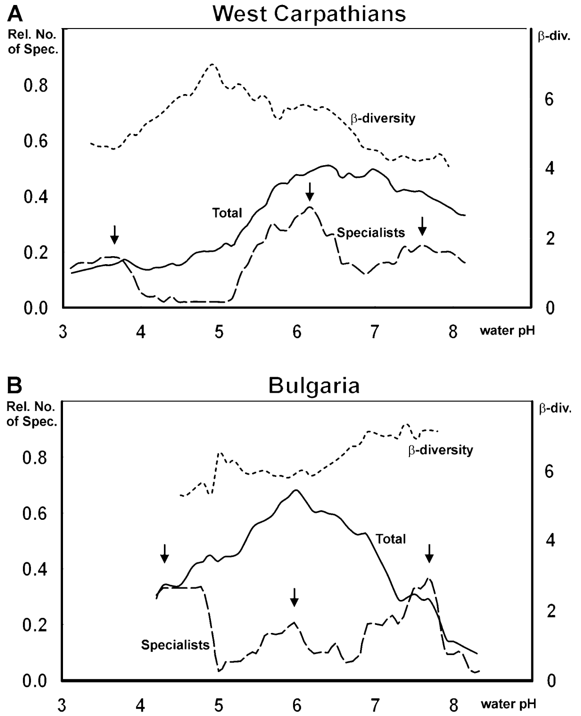

Fig. 3. The relative number of species (percentage of all analysed species) whose tolerances overlap with the given pH value rounded at 0.1 unit. The curves represent the relative number of specialists, i.e. first third of the species sorted by increasing response interval, and the relative number of all species in the analysis. The uppermost curve shows the changes of Whittaker's beta-diversity index along the target gradient. (A) West-Carpathian mires and pH; (B) Bulgarian mires and pH.

Discussion

Evolutionary species pool hypothesis

These data broadly support the ESPH prediction that more species are found in more contemporarily common habitats (Taylor et al. 1990, Schamp et al. 2002, 2003, Pither and Aarssen 2005a). The shape of the pH distributions in both the West Carpathians and Bulgaria corresponds to the shape of the species richness–pH relationship for each region. While only one of two distribution tests found that the West Carpathian pH distribution was significantly 'hump'-shaped, this most likely results from the extreme left-skew of this relationship such that higher pH habitats are much more common than intermediate pH habitats. Indeed, the richness–pH relationship for this region was strongly linear-increasing (Table 1). In both regions, high mire species richness occurred in circumneutral pH mires, which were correspondingly the most common. The richness–pH relationship in Bulgarian mires was better predicted by the quadratic component of a polynomial regression, and possessed a much more internal peak in richness than did the relationship for the West Carpathians. Correspondingly, and in further support of the ESPH, tests confirmed a strong 'hump'-shaped pH distribution in Bulgaria such that intermediate pH communities were most common and were characterized by the highest species richness.

Other authors have advocated a greater subdivision of the environmental gradient to determine the influence of habitat commonness (Pither and Aarssen 2005a) and the inclusion of 'commonness' in regression models. Such an approach may be more appropriate when patterns of habitat commonness for a single environmental factor (i.e. pH) are expected to be a strong determinant of species richness relative to other factors. For mire vegetation, we contend that while habitat commonness is an important component in determining species richness, it is most likely one of several determinants. The commonness of other, potentially important environmental variables may also be important, and have contrasting contemporary and historical distributions of commonness. Species richness within mires can be further influenced, for example, by altitude (Hájková et al. 2006), organic matter levels in peat (Hájková and Hájek 2003), accumulated litter (Hájková and Hájek 2003, Peintinger and Bergamini 2006) and/or nutrient availability (Güsewell et al. 2005) and its fluctuation (Navrátilová et al. 2006). A relationship between productivity and species richness in fens has also been demonstrated (Wheeler and Shaw 1991), but this relationship is very weak for both regions examined here (see Hájková and Hájek 2003 for the West Carpathains). Given that pH explains relatively little variance in species richness in our data sets (Fig. 2), we may not expect the evolutionary history of pH commonness to be as important in driving patterns in these regions. Finally, we acknowledge that contemporary distributions of habitat pH may not reflect historical patterns perfectly. Details of habitat history may have contributed to variability in the relative commonness of parts of the pH distribution through time (see discussion of refugia below). Such variation through time will inevitably obscure the influence of habitat commonness when assessed using the contemporary distribution of habitat pH (Schamp et al. 2002, 2003). For these reasons, we believe our test reveals a significant influence of habitat commonness on patterns of species richness in mires for both West Carpathian, and Bulgarian regions. We encourage further exploration of the influence of habitat commonness on species pools, and contemporary patterns of diversity.

The role of specialists

Our data provide clear, cross-validated evidence for the existence of new non-trivial pattern of plant species richness in mires: the uneven distribution of habitat specialists along pH gradients in two regions. We consider our findings robust with respect to potential confounding factors. Neither beta diversity nor the regional pH distribution pattern matches the observed distribution of specialists (Fig. 3). The fact that omitting or including species with ecological optima outside mire vegetation did not alter the results, suggests that vicinism does not play a significant role. An identical pattern was obtained when the interquartile range was used as a measure of species tolerance instead of the response interval calculated by HOF models. We confirmed the three-peaked distribution of specialists using the conventional phytosociological method of calculating fidelity (Chytrý et al. 2002). Fidelity expresses the diagnostic value of the species for a particular group of samples, i.e. for a given pH class in our case. Narrow-niche species have high fidelity to certain pH classes. When we subdivided the pH gradient by the interval 0.1 pH unit and then calculated fidelity as a probability of non-random occurrence (Fischer's exact test), the number of species with a significant link to the pH class peaked at the same three parts of the pH distribution as they did using our other analyses with HOF models.

It has recently been suggested that determining whether species are specialists requires a null model (Pither and Aarssen 2005b). The null model employed by Pither and Aarssen (2005b) assumes that dispersal is not limited between study sites. While this assumption may apply for the diatom communities studied by Pither and Aarssen (2005b), it is most certainly unrealistic when applied to regional distributions of plant species. This problem was confirmed when we explored our data using this null model and found that nearly all species in the West Carpathians were classified as having significantly narrower niches than expected by chance (i.e. they were specialists). While a spatially explicit version of this model would certainly address this issue (advocated by Pither and Aarssen 2005b), to be objective it would require detailed dispersal information for all species in each region, which is presently unrealistic. Lastly, when we examined species-specific p-values generated using this model as a measure of relative specialisation, we observed similar patterns of relative specialization found using HOF models (i.e. strong specialists were strong specialists in both models) lending further support to our results.

Contrary to our expectations, the distribution of habitat specialists along the pH gradient in mires is not bimodal, like the general distribution pattern of pH in the West Carpathians, but instead has three distinct peaks. One possible explanation is that a bimodal distribution of pH in mires is not consistent across different regions in spite of the two hypothesized buffer systems (Gorham and Janssens 1992, Wheeler and Proctor 2000), and that three modes have been common through evolutionary time. While Sjörs and Gunarsson (2002) reported the second mode of pH distribution around pH 6, other authors report this mode at circumneutral pH values (Gorham and Janssens 1992, Vitt 2000, Hájková and Hájek 2004, this study). Variation in water aeration can dramatically alter the results of pH measurements and resulting pH distributions (Tahvanainen and Tuomaala 2003). The second mode in pH distributions for which pH was measured using unaerated samples of mire water typically occurs at pH values near 5.8, while measuring pH with aerated water samples leads to finding the second mode at circumneutral pH values between 7.2 and 7.6. This difference can be caused by the sampling protocol used (Tahvanainen and Tuomaala 2003), but also by natural water movement near the springs (Sparling 1966, Hájková et al. 2006). The distribution of pH-specialists in mires may therefore reflect species adaptations to the three most historically common pH classes in general: acidic, circumneutral and alkaline. Nevertheless, both West Carpathian and Bulgarian regions have bimodal distributions of conductivity (not shown). While a bimodal distribution of pH can result from the activity of different buffer systems combined with variable water dynamics, water conductivity distributions are instead a reflection of total mineral richness. Therefore, uneven distributions of pH and conductivity in mires are likely caused by three predominant types of mire water chemistry: (1) ombrotrophic; (2) minerotrophic on crystalline bedrock, where the calcium concentration rarely exceeds 10 mg l^{-1} (Sjörs 1952, Malmer et al. 1992, Tahvanainen 2004); (3) minerotrophic on calcareous bedrock, where the calcium concentration is rarely less than 80 mg l^{-1} (Boyer and Wheeler 1989, Hájek et al. 2002, Johnson and Steingraeber 2003). This tripartition of habitats by water chemistry may also be used to explain the trimodal distribution of pH-specialists along pH gradients in both Carpathian and Bulgarian regions.

Historical interpretation of the trimodal specialist distribution

The comparison of the data sets from the West Carpathians and Bulgaria shows a marked difference in the position of acidic peaks: ca 3.6 vs ca 4.7, respectively. This fact does not fit well the hypothesis that the trimodal pattern of pH-specialist distribution is driven by hydrochemical buffer processes. Furthermore, true ombrotrophic bogs do not occur in Bulgaria at all

(Hájková et al. 2006). The origin of the acidic peak of specialists in Bulgaria then requires another explanation.

We hypothesize that the pattern of specialist distribution reflects the refugial history of mire habitats in both geographic regions. During the pleniglacials in Bulgaria, extremely dry climate predominated and *Ephedra* steppes reached very high altitudes (Tonkov et al. 2002) leaving few places where mire flora could survive (Natcheva and Cronberg 2003). During this time period, mire vegetation mainly survived in wetlands that occurred along lakes or streams high in the crystalline mountains (Rila, Pirin), and at the margins of extremely calcium-poor lakes in the Rhodope Mts. In these regions, *Sphagnum* spores are documented from ca 10 000 years BP (Huttunen et al. 1992, Tonkov et al. 2002). Thus, the predominance of acidic, non-calcareous substrate in refugial areas of the Bulgarian high mountains may be responsible for the apparent shift in pH-optima of rich-fen species towards more acidic conditions (Hájková et al. 2007). This corresponds to the hypothesis of Pither and Aarssen (2005a) who anticipate the existence of local adaptations of species in regions differing in the abundance of acidic and higher-pH substrates. Tyler (2003) noted that calcifuges respond to a toxic aluminium signal at their root surfaces in acidic soils by exuding compounds that may be similar to, or identical with compounds exuded by calcicoles in calcareous soils. Therefore, a switch-over between adaptation to acidic and calcareous substrates may not require a complicated reorganization of the genome. This assumption is further supported by the analysis of Prinzing et al. (2001) who demonstrated that the variance in species niche position along a pH gradient can be better explained at the level of species than genera or family. Specialist adaptation to acidity may therefore be the result of relatively young evolutionary processes (Chytrý et al. 2003). In the light of these facts, the disparate position of the acidic peaks in habitat specialist richness in both the West Carpathians and Bulgaria may be interpreted as an example of the importance of ecological conditions in refugia in driving the evolution of species' habitat requirements (Ewald 2003).

The two other peaks in specialist richness along the pH gradient overlap for the two study regions and may be interpreted in terms of historical refugial patterns as well. Other habitats where mire flora could survive dry periods in Bulgaria include fens on artesian springs below the limestone coat of the Stara Planina Mts, and scattered tufa-forming spring fens. This hypothesis is supported by the unique occurrences of many plant and snail species in these habitats (Hájek et al. 2005, Horsák unpubl.), and consequently, these extremely alkaline habitats harbour species which are responsible for the ''alkaline'' peak of specialist richness. The alkaline peak in the West Carpathians is formed by species found in these same habitats – basin calcareous fens and travertine springs – which have also acted as refugia for mire flora (Grootjans et al. 2005).

The Bulgarian subneutral peak (5.8–6.0) is less distinct compared to that of West Carpathians, possibly due to the lower number of subalpine–spring species, which were ranked as only ''moderate specialists'' in Bulgaria (Appendix 1) and due to the relative rarity of their habitats.

The age of mire habitats and pH gradient

Our historical interpretation may be questioned on the basis of palaeoecological data that suggest a relatively young origin of ombrotrophic bogs compared to both subneutral brown-moss fens and calcareous fens (Rybníček and Rybníčková 1968, Ralska-Jasiewiczowa 1980, Kuhry et al. 1993). This implies that the number of species adapted to acidic conditions should be lower than those adapted to base-rich conditions (Pärtel 2002, Pither and Aarssen 2005a). Ombrotrophic bogs really have low local species richness and their flora represents only several plant families (*Vacciniaceae, Ericaceae, Sphagnaceae* and few others). Nevertheless, when we focus on pH-specialists, we find that nearly the entire species pool of ombrotrophic bogs is composed of pH-specialists and that their number is similar to the number of high-pH specialists. If we interpret this fact in the historical context, we can assume that in the West Carpathians the history of bogs could be similar to the history of fens. Very old findings of *Sphagnum* spores as compared to other central-European regions (about 10 000 years BP, Gajewski et al. 2001) suggest that at least small ombrotrophic hummocks occurred during the Pleistocene (Bellamy and Rieley 1967). Active ombrotrophic bogs gradually rise up towards the phase when the climate is no longer able to maintain a stable water regime in such a large peat body and at this point a bog starts to desiccate. It is likely that active bogs during Pleistocene have not survived till present and therefore we have a biased estimate of the age of ombrotrophic habitats. The recent finding of ombrotrophic-bog sediments overlayed by calcareous-fen deposits and dated to 10 000 years BP in the western Carpathians (Grootjans et al. 2005, Hájková, unpubl.) support this possibility.

Conclusions

Our findings support the prediction of the ESPH that more common habitats will host a greater number of species (Taylor et al. 1990, Schamp et al. 2002, 2003, Pither and Aarssen 2005a). This lends further support to the ESPH prediction that habitat commonness will play an important role in shaping regional species pools, and subsequently influencing contemporary patterns of species richness. These findings also extend the existing evidence for the ESPH to include mire habitats. An examination of the distribution of specialists, however, reveals that correspondence between habitat commonness and species pool size can not completely reveal how historical processes are related to the contemporary distribution and diversity of plant species in West Carpathian and Bulgarian mires. Region-specific historical commonness of habitats with respect to pH may drive patterns in the richness of pH specialists. We have demonstrated that the distribution of specialists along ecological gradients may be independent from overall species richness and total species pool size and that acid mires have the same number of pH-specialist plant species as calcareous mires, even if the total species pool is larger in the latter. These results can be compared to those found by Lennon et al. (2004) who found that more common bird species are most responsible for richness patterns. Similarly, Pärtel et al. (2001) found that alpha diversity for plant species was determined by common species and beta diversity by rare species. For this reason, the importance of historical habitat commonness may be obscured when researchers do not distinguish between specialists and generalists. The refugial and evolutionary history of mires appears to be much better related to the distribution of pH-specialists along the pH-gradient than to overall species richness patterns.

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Appendix 1. A list of species whose tolerance falls within the observed peaks of pH-specialists distribution. Bryophytes are marked by the abbreviation “(b)”.

Western Carpathians

acidic peak	subneutral peak	alkaline peak
3.5–3.7	6.0–6.2	7.4–7.6

strong specialists (are among 50 species with narrowest responses)

Calluna vulgaris	*Blindia acuta** (b)	*Carex distans*
Drepanocladus fluitans (b)	*Calliergon giganteum* (b)	*Carex flacca*
Empetrum hermaphroditum	*Cardamine opizii**	*Carex hostiana*
Sphagnum fuscum (b)	*Carex chordorrhiza*	*Carex paniculata*
Sphagnum magellanicum (b)	*Drepanocladus exannulatus* (b)	*Cratoneuron commutatum* (b)
Sphagnum rubellum (b)	*Paludella squarrosa* (b)	*Cratoneuron filicinum* (b)
Vaccinium microcarpum	*Philonotis seriata** (b)	*Schoenoplectus tabernaemontani*
Vaccinium myrtillus	*Poa alpina**	*Polygala amarella*
Vaccinium uliginosum	*Pohlia wahlenbergii** (b)	*Salix repens ssp. rosmarinifolia*
Vaccinium vitis-idaea	*Ranunculus flammula*	*Taraxacum Sect. Palustria*
	*Rhizomnium punctatum agg.** (b)	*Valeriana dioica*
	*Scapania irrigua** (b)	
	*Scapania undulata** (b)	
	*Silene pusilla**	
	Sphagnum contortum(b)	
	Sphagnum teres (b)	
	Sphagnum warnstorfii (b)	

moderate specialists (are among 80 species with narrowest responses)

Eriophorum vaginatum	*Aulacomnium palustre* (b)	*Carex lepidocarpa*
Polytrichum strictum (b)	*Calliergon stramineum* (b)	*Dactylorhiza incarnata*
Sphagnum compactum (b)	*Carex lasiocarpa*	*Eleocharis uniglumis*
	Carex viridula	*Equisetum telmateia*
	Cratoneuron decipiens (b)	*Gymnadenia densiflora*
	*Dicranella palustris** (b)	*Philonotis calcarea* (b)
	Geum rivale	*Tofieldia calyculata*
	Hypnum lindbergii (b)	*Triglochin palustris*
	Hypnum pratense (b)	
	Pedicularis palustris	
	Philonotis fontana (b)	
	Potentilla palustris	
	Salix pentandra	
	Sphagnum flexuosum (b)	
	Sphagnum subnitens (b)	
	Sphagnum subsecundum (b)	
	Tephroseris crispa	
	Viola palustris	

Bulgaria

acidic peak	subneutral peak	alkaline peak
4.6–4.8	5.8–6.0	7.5–7.7

strong specialists (are among 30 species with narrowest responses)

Bruckenthalia spiculifolia	*Hamatocaulis vernicosus* (b)	*Blysmus compressus*
Drosera rotundifolia	*Philonotis caespitosa* (b)	*Carex distans*
Eriophorum vaginatum	*Ranunculus flammula*	*Carex flacca*
Menyanthes trifoliata	*Sphagnum contortum* (b)	*Cratoneuron filicinum* (b)
Polytrichum commune (b)	*Sphagnum platyphyllum* (b)	*Eleocharis uniglumis*
Sphagnum capillifolium (b)	*Veronica scutellata*	*Epipactis palustris*
Sphagnum compactum (b)		*Linum catharticum*
Sphagnum palustre (b)		*Mentha longifolia*
Sphagnum warnstorfii (b)		*Cratoneuron commutatum* (b)
Vaccinium uliginosum		*Philonotis calcarea* (b)
		*Silene pusilla**
		Triglochin palustris

Appendix 1 (*Continued*)

moderate specialists (are among 50 species with narrowest responses)

Calliergon stramineum (b) *Primula deorum**	*Calliergon sarmentosum** (b) *Caltha palustris* *Cardamine balcanica** *Crepis paludosa* *Dicranella palustris** (b) *Equisetum fluviatile* *Myosotis nemorosa* *Philonotis fontana* (b) *Philonotis seriata** (b) *Primula farinosa exigua** *Saxifraga stellaris** *Trichophorum caespitosum**	*Eleocharis quinqueflora*

*species confined to vegetation plots above the timberline in our data set

Appendix 2. A list of species whose tolerance falls within the observed peaks of conductivity-specialists distribution. Water conductivity (μS cm^{-1}) approximates total mineral richness of the water and represents the sum of calcium and magnesium concentrations (ln-transformed for analysis). Abbreviations: *Salix *rosmarinifolia – S. repens* subsp. *rosmarinifolia*; *Sch.tabernaemontani – Schoenoplectus tabernaemontani*. Bryophytes are marked by the abbreviation “(b)”.

Western Carpathians (36 species from 50 specialists)

without minerals	moderately rich	calcareous	salt-rich
Conductivity (ln) 1.36–1.7	4.42–4.76	6.29–6.33	7.82–8.16
Conductivity 4.0–6.0	83–117	539–757	2490–3498
Drepanocladus fluitans (b) *Sphagnum rubellum* (b) *Sphagnum fuscum* (b)	*Calliergon giganteum* (b) *Calliergon stramineum* (b) *Carex chordorrhiza* *Carex pulicaris* *Dicranum bonjeanii* (b) *Drosera anglica* *Hypnum lindbergii* (b) *Lotus pedunculatus* *Paludella squarrosa* (b) *Potentilla palustris* *Ranunculus flammula* *Sphagnum contortum* (b) *Sphagnum flexuosum* (b) *Sphagnum subnitens* (b) *Sphagnum subsecundum* (b) *Sphagnum teres* (b) *Sphagnum warnstorfii* (b) *Viola palustris*	*Carex flacca* *Carex hostiana* *Carex lepidocarpa* *Carex paniculata* *Cratoneuron commutatum* (b) *Trichophorum pumilum* *Equisetum telmateia* *Linum catharticum* *Mentha longifolia* *Philonotis calcarea* (b) *Salix *rosmarinifolia*	*Blysmus compressus* *Carex distans* *Eleocharis uniglumis* *Sch.tabernaemontani*

Bulgaria (24 species from 30 specialists)

without minerals	moderately rich	calcareous
conductivity (ln) 2.98–3.3	4.2–4.5	5.6.–6.0
conductivity 20–27	69–94	282–387
Calliergon sarmentosum (b) *Dicranella palustris* (b) *Polytrichum commune* (b) *Primula deorum* *Sphagnum capillifolium* (b) *Trichophorum caespitosum*	*Eriophorum angustifolium* *Geum rhodopaeum* *Drepanocladus vernicosus* (b) *Oenanthe banatica* *Oenanthe fistulosa* *Philonotis caespitosa* (b) *Potentilla palustris* *Ranunculus flammula* *Veronica scutellata*	*Blysmus compressus* *Carex distans* *Carex flava* *Cratoneuron filicinum* (b) *Eleocharis uniglumis* *Gratiola officinalis* *Linum catharicum* *Cratoneuron commutatum* (b) *Philonotis calcarea* (b)