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Investigating niche and lineage diversification in widely distributed taxa: phylogeography and ecological niche modeling of the *Peromyscus maniculatus* species group

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The relationship between lineage formation and variation in the ecological niche is a fundamental evolutionary question. Two prevailing hypotheses reflect this relationship: niche conservatism and niche divergence. Niche conservatism predicts a pattern where sister taxa will occupy similar niche spaces; whereas niche divergence predicts that sister taxa will occupy different niche spaces. Widely distributed species often show distinct phylogeographic structure, but little research has been conducted on how the environment may be related to these phylogenetic patterns. We investigated the relationship between lineage divergence and environmental space for the closely related species *Peromyscus maniculatus* and *P. polionotus* utilizing phylogenetic techniques and ecological niche modeling (ENM). We estimated the phylogenetic relationship among individuals based on complete cytochrome *b* sequences that represent individuals from a majority of the species ranges. Niche spaces that lineages occupy were estimated by using 12 environmental layers. Differences in niche space were tested using multivariate statistics based on location data, and ENMs were employed using maximum entropy algorithms. Two similarity indices estimated significant divergence in environmental space based on the ENM. Six geographically structured lineages were identified within *P. maniculatus.* Nested within *P. maniculatus* we found that *P. polionotus* recently diverged from a clade occupying central and western United States. We estimated that the majority of the genetic lineages occupy distinct environmental niches, which supports a pattern of niche divergence. Two sister taxa showed niche divergence and represent different ecomorphs, suggesting morphological, genetic and ecological divergence between the two lineages. Two other sister taxa were observed in the same environmental space based on multivariate statistics, suggesting niche conservatism. Overall our results indicate that a widely distributed species may exhibit both niche conservatism and niche divergence, and that most lineages seem to occupy distinct environmental niches.

A fundamental question in speciation concerns the influence of the ecological niche on lineage divergence. Most of the current discussion on the relationship between lineage divergence and the ecological niche is dominated by two prevailing hypotheses: niche conservatism and niche divergence. Niche conservatism predicts that closely related taxa retain ancestral ecological affiliations and persist in similar environments. This may be caused by stabilizing selection or lack of variation in ancestral traits (Lord et al. 1995, Webb et al. 2002, Wiens and Graham 2005), but niche conservatism is primarily a pattern of evolution and by itself does not explain causality (Losos 2008). Empirical work has shown that divergence of allopatric sister taxa is often characterized by niche conservatism, whereby geographic isolation influences the pattern of speciation without shifts in niche dimensions (Peterson et al. 1999, Peterson 2001, Kozak and Wiens 2006). The alternative hypothesis, niche divergence, predicts that sister taxa will occupy different niches. Under this hypothesis, being adapted to different environmental conditions can promote lineage divergence, even in sympatry. Evidence is accumulating that sister taxa often exhibit niche divergence and that niche evolution may be more common than initially assumed (Losos et al. 2003, Graham et al. 2004, Pyron and Burbrink 2009, Dormann et al. 2010). Differences in niche space can be observed in recently diverged taxa, as researchers have found that niches can shift in as little as 10^4–10^5 yr (Evans et al. 2009). In these cases, the environment may function as a barrier to gene flow if two or more allopatric lineages are separated by suboptimal conditions (Rissler and Apodaca 2007). Alternatively, adaptation to different local and regional environmental conditions may prevent lineages from interacting, thus reducing gene flow even when distributed in sympatry or parapatry (Wiens and Graham 2005).

Widely distributed species are ideal for studying the relationship between lineage divergence and niche divergence. Such taxa often show patterns of genetic or phenotypic structure along environmental gradients, as heterogeneous

landscapes tend to foster adaptation to distinct niches (Avise et al. 1987). In North America and Europe, many widespread species are divided into distinct lineages that have either allopatric or parapatric distributions (Taberlet et al. 1998, Hoffman and Blouin 2004, Fontanella et al. 2008). However, little attention has been given to the possible influence that the environment might have on the diversification and maintenance of these lineages.

Phylogeography has been widely used to identify diverging lineages in a spatial context (Lansman et al. 1983) and is currently the most utilized tool for investigating the connection between micro- and macroevolutionary processes related to speciation (Hickerson et al. 2010). This approach identifies evolutionary relationships and the spatial component of the formation and maintenance of biodiversity (Avise et al. 1987, Avise 2000). This spatial component has usually been limited to geographic distances, which unfortunately ignores a great deal of environmental complexity that may impact taxa (Graham et al. 2004). Recent studies have combined phylogeography and ecological niche modeling (ENM) to understand the relationship of distributions and niche spaces with speciation and the maintenance of genetic variation (Graham et al. 2004, Rissler and Apodaca 2007, Jakob et al. 2009, Pyron and Burbrink 2009). Ecological niche modeling for taxa with wide distributions can help identify contact zones between lineages, quantify their spatial overlap, and explore the possible nature of their isolation (Kozak et al. 2008).

Our application of ENM parallels species distribution models (SDM), and predicts spatial distributions of taxa based on occurrence records and environmental data (Elith and Leathwick 2009). The predicted spatial distribution gives an estimate of the ecological niche: the multi-dimensional environmental space that bounds a species' persistence (Hutchinson 1978). Utilizing the ENM approach facilitates the testing of correlations between lineage diversification and the environmental setting those lineages occupy.

To test for the association between environmental conditions and lineage divergence, we used the widely distributed mammal species *Peromyscus maniculatus* and the more narrowly distributed *P. polionotus* as a model system. These taxa belong to a species group first defined by Osgood (1909) and are distributed throughout North America (Hall 1981, Carleton 1989). Diverse habitat conditions occur over the geographic distribution of *P. maniculatus*, which may have led to isolation of populations. Phylogeographic lineages have previously been identified in *P. maniculatus* (Lansman et al. 1983, Dragoo et al. 2006, Gering et al. 2009), and morphotypes associated with different habitats have also been observed (Blair 1950, Carleton 1989). Morphological variation is further reflected in the recognition of 67 subspecies within *P. maniculatus* (Hall 1981). The closely related species *P. polionotus* is restricted to the southeastern United States, where it is hypothesized to have diverged from a grassland form of *P. maniculatus* (Carleton 1989). Until recently, limited data were available to address the origin of *P. polionotus*. Through the efforts of this and other research (Dragoo et al. 2006, Degner et al. 2007, Van Zant and Wooten 2007, Gering et al. 2009), we compiled a comprehensive dataset for these two species to evaluate the evolutionary relationships of these taxa and to explore the relationship of environment to lineage divergence between and within these species. Thus we: 1) inferred phylogeographic relationships among individuals of *P. maniculatus* and *P. polionotus*, 2) established whether phylogeographic lineages occupy distinct climatic niches, and 3) predicted distributions of each lineage to determine potential overlap and contact zones.

Material and methods

Taxon sampling

We assembled a cytochrome *b* (cyt *b*) data set using 478 samples that were obtained throughout the known range of *P. maniculatus* and *P. polionotus*. A total of 343 *P. maniculatus* (DQ385628-DQ385827; EF666142-EF666277; EU006766-EU006772), 38 *P. polionotus* (EF216336-EF216347; EU140757-EU140793), 2 *P. keeni* (DQ385716 and EU140797), and 5 *P. melanotis* (DQ385626 and EU574689-EU574701) sequences were obtained from GenBank (Dragoo et al. 2006, Degner et al. 2007, Van Zant and Wooten 2007, Gering et al. 2009). Additionally, 89 tissue samples of *P. polionotus*, representing 9 populations, were collected from peninsular Florida. Published sequences from two additional species were included as outgroups: *P. gossypinus* (DQ385625; Dragoo et al. 2006), and *P. leucopus* (AF131926; Bradley et al. 2000).

DNA extraction and sequencing

DNA from collected samples was extracted using a DNeasy tissue kit (Qiagen). We amplified the complete mitochondrial (mtDNA) cyt *b* gene using the primers 14724F and TD-20 (Van Zant and Wooten 2007). PCR conditions followed Herron et al. (2004). Sequencing was conducted by the Nevada Genomics Center using an ABI 3730 sequencer and chromatograms were edited using Sequencher v.4.7 (Gene Codes Corp.). Alignments of the sequences were made by visual inspection using GeneDoc v.2.6 (Nicholas et al. 1997). GenBank accession numbers for sequences generated by this study are listed in Supplemental material (Table A1).

Phylogenetic analysis

We identified haplotypes and reduced redundancy in the data with TCS (Clement et al. 2000), which estimates a haplotype network with maximum parsimony. The output includes a list of individuals with the same haplotypes, and only one representative of each haplotype was included in the final alignment. Phylogenetic analyses were conducted using Bayesian inference (BI; MrBayes v.3.1.2; Huelsenbeck and Ronquist 2001) and maximum likelihood (ML; RAxML v.7.0; Stamatakis et al. 2008). We used Bayes factors to determine the best partitioning strategy in BI (Brandley et al. 2005). Preliminary analyses yielded a two-partition model, with 1st and 2nd codon positions together and the 3rd position separate. Substitution models for each partition in BI and ML were identified by MrModeltest v.2.4 using

the Akaike information criterion (Nylander 2004). For BI we initiated two independent Markov Chain Monte Carlo (MCMC) runs, each with four chains, and ran them for 10^7 generations, sampling every 1000 generations. Using Tracer v.1.4 (Rambaut and Drummond 2007), we determined stationarity and conservatively discarded 2×10^6 generations as burn-in. We estimated a ML phylogeny using RAxML v.7.0 (Stamatakis et al. 2008), on the the Cipres Portal v.2.0 (< www.phylo.org/portal2 >). We determined bootstrap values (BS) for ML using 100 pseudo-replicates. We defined lineages based on monophyly and with individuals inhabiting a geographically distinct area (Wiens and Penkrot 2002).

Taxon and environmental sampling for estimating climatic niche

We estimated the niche using two approaches. First we utilized multivariate statistics on climate conditions at lineage occurrence points, which provided information on the climatic niche based on the variables alone. Second we conducted niche modeling, which projects the climatic niche across a geographic region. For both approaches we included the lineages recovered in the phylogenetic analysis of *P. maniculatus* and *P. polionotus* that had more than five location points. We georeferenced our genetic samples based on museum voucher locations, locale descriptions from original papers, and personal communication with the authors of original papers (Dragoo et al. 2006, Degner et al. 2007, Van Zant and Wooten 2007, Gering et al. 2009). Our analysis included only one sample per location.

We utilized climatic variables obtained from the WorldClim database with a resolution of 30 arc-seconds (Hijmans et al. 2005). As temperature and precipitation can impact the metabolic rate of *Peromyscus* species (MacMillen and Garland Jr 1989), climate could provide insight into the spatial distribution of *Peromyscus* species. Additionally, we incorporated an altitude layer from the Shuttle Radar Topography Mission (SRTM) data set, as *Peromyscus* species exhibit different torpor patterns depending upon the elevation they occupy (Tannenbaum and Pivorun 1984).

Some of the environmental variables are highly correlated and could over-parameterize the models. To reduce over-parameterization we identified and eliminated correlated variables using the methods of Rissler and Apodaca (2007). We extracted climatic and altitude data across North America for 10^5 randomly generated points; for each pair of variables we estimated Pearson correlation coefficients using JMP v.8.0 (SAS Inst.). For those pairs of variables that exceeded our threshold of $r = 0.75$, we included the variable which was most biologically meaningful. We incorporated 12 variables, one quantifying altitude and the others quantifying variation in temperature and precipitation: annual mean temperature, mean diurnal temperature range, isothermality, annual temperature range, mean temperature of wettest quarter, mean temperature of coldest quarter, seasonal precipitation, precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, and precipitation of coldest quarter.

Estimation of climatic niche by occurrence points

We tested for significant differences in the climatic niche based on occurrence points of all lineages using two methods. First, we used parametric multivariate statistics to compare the multi-dimensional climatic values between sample locations. We extracted climatic and altitude values for each georeferenced genetic sample using ArcGIS v.9.2 (ESRI, Redlands, CA, USA). We square-root transformed those variables not found to be normally distributed: altitude, mean diurnal temperature range, precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, and precipitation of coldest quarter. We evaluated homogeneity of variances based on Levene's test conducted in SPSS v.18.0, and all variables met this assumption. Overall significant differences among the climatic spaces that the lineages occupy were determined using a multivariate analysis of variance (MANOVA) in SPSS v.11.0.

As a second approach to compare climatic niches among lineages, we used parametric discriminant analysis (DA). Discriminant analysis maximizes explained variation based on a priori defined groups and ordinates the variables at occurrence points. Ordination reduces the number of variables needed to explain the variation among the groups, and removes collinearity among ordination axes for subsequent analysis. For the DA analyses, groups were defined as the lineages inferred by phylogenetic analysis. Canonical scores (CV) for the DA were determined in JMP v.7.0. To determine differences in CV among lineages, we estimated the centroid and 95% confidence interval (CI) for each lineage. Lack of overlap of the 95% CI in at least one of the CV axes suggested deviation in the environmental space we estimated for the lineages.

Testing for spatial autocorrelation

Differences in environmental space among phylogenetic lineages can be the result of spatial autocorrelation. We accounted for this potential bias by performing a partial Mantel test to assess the correlation between environmental and genetic differences while controlling for geographic distance. Environmental differences that were positively correlated with genetic divergence, independent of geographic distance, would suggest that genetic divergence is truly associated with changes in the environment. Statistical significance was estimated based on 999 permutations, with $\alpha = 0.05$, using the vegan v.1.13 package in R v.2.6 (Oksanen 2009). Environmental differences were estimated as the Euclidian distance of CVs among sample locations. We calculated genetic distance with MEGA v.4.0 (Tamura et al. 2007) using a Kimura-2-parameter model and gamma-distributed rate variation. Geographic distance was estimated using the package fields v.4.1 in R (Fields Development Team 2006).

Estimation of climatic niche by niche modeling

The analyses above are limited to point locations, therefore we used a niche model to project the environmental space utilized by a lineage and to test for environmental and geographic association among and between phylogenetic

lineages. We created niche models with Maxent v.3.2, which estimates distributions based upon niche characteristics using a maximum entropy algorithm (Phillips et al. 2006, Phillips and Dudík 2008). Maximum entropy is useful for estimating distributions when absence data are lacking (Phillips et al. 2006) and generally performs better than other distribution modeling approaches (Elith et al. 2006, Wisz et al. 2008). Maxent incorporates a method of regularization for selecting environmental variables when building models. This reduces the need to select environmental variables to avoid over-parameterization, yet some variable selection is recommended to reduce collinearity (Elith et al. 2011). To increase the sample size for creating the model, we included additional locations from natural history museums. Locations were provided by Field Museum of Natural History, Cornell Univ., Florida Museum of Natural History, Los Angeles County Museum of Natural History, Louisiana State Univ. Museum of Zoology, Museum of Southwestern Biology, Mississippi State Univ., Museum of Vertebrate Zoology, Paleobiology Database, Santa Barbara Museum of Natural History, Univ. of Alaska Museum of the North, Univ. of Colorado Museum of Natural History, Univ. of Washington, and Yale Peabody Museum (accessed through GBIF data portal, <www.gbif.net>, 20 April 2009). A minimum convex polygon (MCP) was created using known occurrence points from each phylogenetic lineage, and museum specimens for locations within each MCP were assigned to the appropriate lineage. A GIS shape file of the locations used in the analysis is available upon request.

The occurrence data were randomly partitioned into training (75%) and test (25%) datasets to evaluate the accuracy of each model. We determined model accuracy by calculating the area under the curve (AUC) of the receiver operating characteristic (ROC) plot (Phillips et al. 2006). In Maxent the AUC is the probability, ranging from 0 to 1, that a random presence location is ranked above a random background site (Phillips et al. 2006). A value of 1 implies a perfect fit, 0.5 no different than random, and < 0.5 suggests the model performs worse than random expectation. Models with AUC values above 0.75 are considered good, and models with AUC > 0.90 are considered excellent (Swets 1988, Elith 2002). We determined the best model for each lineage in Maxent by running iterations until the probability of change was 1.0×10^{-5}, or after a maximum of 500 iterations. To identify contact zones and quantify levels of sympatry or parapatry, we estimated the spatial overlap in predicted distributions between all genetic lineages included in the analysis. We used the minimum training presence, which is the minimum probability of occurrence in the modeled distribution, for each model as the threshold to create maps of suitable climatic niche for each lineage. Overlap between two lineages was calculated as the proportion of cells where conditions were predicted to be suitable for both lineages. All estimates of overlap were conducted using ArcGIS v.9.2.

We utilized the niche equivalency method proposed by Warren et al. (2008) to determine differences in the niche between lineages based on entire distributions. We first measured niche overlap between distributions by calculating two indices: Schoener's *D* (Schoener 1968), and *I* (Warren et al. 2008). Both measures give pair-wise niche overlap with values ranging from 0 (no overlap) to 1 (identical niche models). Then, we used the niche equivalency test in ENMtools (Warren et al. 2008) to determine niche differences. This is a one-tailed test which determines if niche models are significantly dissimilar from random by comparing the observed *D* and *I* to a null distribution. The null distribution is derived from pseudo-replicate distributions by randomly assigning lineage membership to the occurrence data for two lineages. Subsequently, *D* and *I* are estimated for the resulting models. This is repeated several times to create the null distribution for *D* and *I* (Warren et al. 2008). We compared all pair-wise combinations of lineages to assess differences in both sister and non-sister taxa. We calculated *D* and *I* for each comparison using 100 pseudo-replicates. Because the spatial scale of this study caused computational constraints in estimating the null distributions of *D* and *I*, we reduced the spatial resolution of the environmental layers to 2.5 minutes when inferring the pseudo-replicate niche models as suggested by Pyron and Burbrink (2009). However, changing the scale of the environmental layers has the potential to significantly change the estimated models (Guisan et al. 2007). To ensure that changing the resolution of the environmental layers did not result in overestimating niche similarity of the null distribution, we estimated *D* and *I* between each lineage for niche models created using the lower resolution climatic and altitude data.

Results

Phylogenetic analysis

The cyt *b* sequence alignment consisted of 1154 base pairs with 274 (23.7%) parsimony informative characters. The sample consisted of 350 haplotypes representing the majority of the geographical distribution of *P. maniculatus* and *P. polionotus*. We identified 13 new cyt *b* haplotypes from the 89 *P. polionotus* individuals sampled for this study (Supplementary material Table A1).

GTR + I + G was the optimal nucleotide substitution model for both partitions as determined by MrModeltest. BI and ML methods produced similar topologies, but ML did not resolve deeper divergences in the phylogeny. We identified 6 lineages within *P. maniculatus* (Fig. 1) that were distinct monophyletic groups located in geographically distinct areas. Lineage 1 was a well supported clade (posterior probability (PP) = 1.00; bootstrap (BS) = 70) associated with the Pacific coast and Rocky Mountains (lineage 1; Fig. 1, 2), and its sister lineage was restricted to the grasslands of the central United States (lineage 2; Fig. 2). Lineage 2 was less well supported (PP = 0.65; BS = 74; Fig. 1), but due to their allopatric distributions we consider lineages 1 and 2 as distinct. Two strongly supported sister lineages were identified in northeastern North America (lineage 3; PP = 0.99; BS = 86; lineage 4; PP = 1.00; BS = 77; Fig. 1). Clades 3 and 4 overlap in part of their ranges based on sample locations (Fig. 2). Lineage 5 was identified in southern New Mexico based on a limited number of sequences (PP = 1.00, BS = 98; Fig. 1). An additional clade was inferred by BI that extends from the Baja California peninsula to California and Nevada (lineage 6; PP = 1.00; BS < 50; Fig. 1, 2).

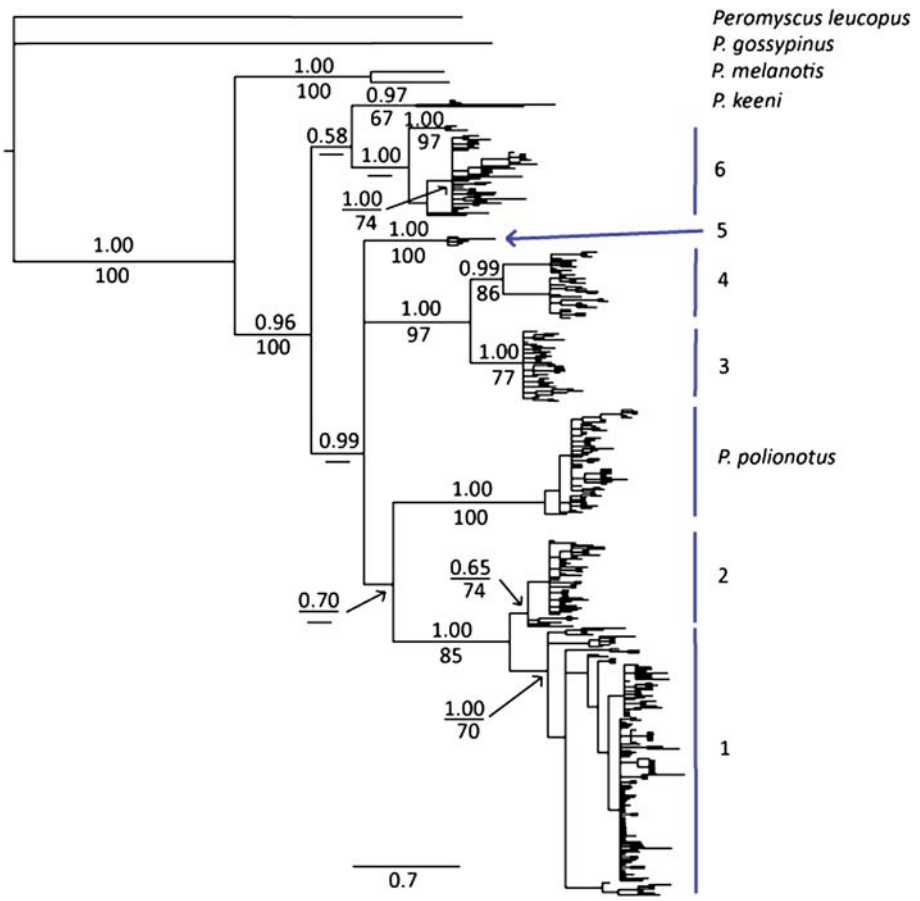

Figure 1. Phylogram based on Bayesian inference of cyt *b* sequences of the *P. maniculatus* species group. Nodal support given by Bayesian posterior probability (PP) above the line, and maximum likelihood bootstrap (BS) below line. Values below 0.5 indicated by a dash (–). Numbers indicate lineages of interest within *P. maniculatus*.

We determined that two of the other members of the species group, *P. polionotus* and *P. keeni*, rendered *P. maniculatus* paraphyletic. *Peromyscus keeni* formed a clade based on BI (PP = 0.97). This lineage seems to be associated with the western lineages (lineage 6), but the relationship is poorly supported (PP = 0.58; BS < 50). *Peromyscus polionotus* forms a strongly supported clade (PP = 1.00; BS = 99) that is nested within *P. maniculatus*. Both BI and ML place *P. melanotis* as the sister species to the remaining members of the *P. maniculatus* species group (PP = 1.00; BS = 100).

Estimated climatic niche and niche modeling

We modeled *P. polionotus* and the five most widespread lineages of *P. maniculatus* (lineage 1, 2, 3, 4 and 6; Fig. 2). Lineage 5 was only identified by 4 sample locations, covering an area of just over 7 km^2. With such limited spatial information we did not include this lineage in the niche modeling because extremely small sample numbers may not produce accurate models (Hernandez et al. 2006, Wisz et al. 2008).

Among the sample locations of the six lineages included in our analysis, we found a significant overall difference in the climatic and altitude variables at occurrence points (MANOVA, Wilks' lambda = 0.007, $F_{60, 542} = 17.4$, $p < 0.001$). Discriminant analysis indicated 93.4% of environmental variation among the genetic lineages was explained by the first two canonical scores (CV; Fig. 3). The first axis explained 74.3% of the variation and was primarily determined by mean diurnal temperature range. The second axis explained 19.1% of the variation and was determined mainly by precipitation of the driest quarter and precipitation of the warmest quarter. On the first axis, 95% confidence intervals overlap for only lineages 3 and 4 (Fig. 3). Along the second axis, lineages 1, 2 and 6 formed a group, separate from a second group where lineages 3 and 4 overlap. The *P. polionotus* lineage remained separate on the second axis (Fig. 3). Discriminant analysis supports the MANOVA result of separation in the environmental space among the lineages. Overall the lineages differ in their environmental space, based on the canonical scores. The only lineages without differentiation on either axis were lineages 3 and 4.

Among the lineages we found a positive correlation between genetic distance and environmental distance when geographic distance was controlled ($r = 0.562$; $p = 0.001$; Table 1). We also observed a significant positive correlation within lineages 3 and 4 (lineage 3, $r = 0.412$, $p = 0.020$; lineage 4, $r = 0.272$, $p = 0.004$; Table 1). Within the remaining lineages (1, 2, 6 and *P. polionotus*) we found no correlation between genetic and environmental distance when we controlled for geographic distance (Table 1).

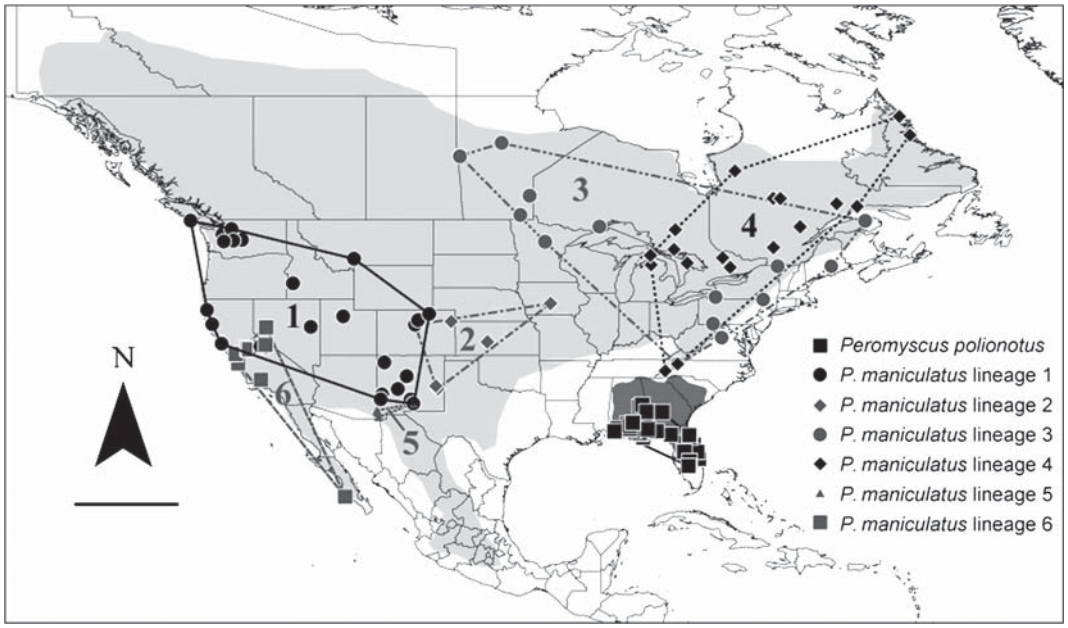

Figure 2. Spatial distribution of *P. polionotus* and six monophyletic lineages of *P. maniculatus* based on phylogenetic analysis of cyt *b*. Distribution for the two species shown as shaded area: *P. maniculatus* in light grey and *P. polionotus* in dark grey. Scale bars equal 1000 km.

We developed distribution models using 189 locations for *P. maniculatus* lineage 1, 45 for lineage 2, 55 for lineage 3, 40 for lineage 4, 107 for lineage 6, and 64 for *P. polionotus*. Each model showed high specificity with AUC values for the test dataset ranging from 0.903 to 0.996. These models exhibited little overprediction outside of the locations included to create the models (Fig. 4). The minimum probability of occurrence for training points ranged from 0.066 for lineage 6 to 0.256 for lineage 2. The most important variables for each model were different. Mean temperature for driest quarter was most important for lineages 2 and 3, while precipitation of the warmest quarter was most important for lineage 6 and *P. polionotus*. The remaining models had the following greatest weight of variables: lineage 1 (isothermality), and lineage 4 (precipitation of driest quarter). For each model we determined that four variables were needed to explain 84% or more of the variation, with the remaining variables providing little information to the predicted distributions.

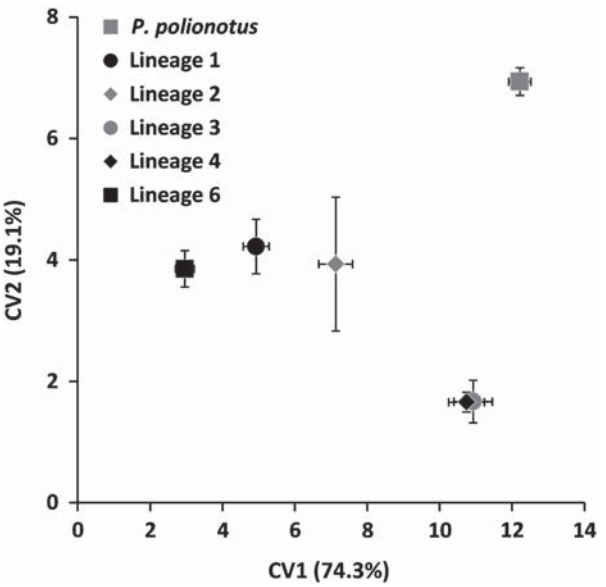

Figure 3. Result of discriminant analysis for testing divergence in environmental space for five phylogenetic lineages of *P. maniculatus* and *P. polionotus*, based on 11 climatic layers and altitude. Percent explained variation for each CV is reported for both axes; total variation explained was 93.3%. Each point represents the centroid for each lineage; error bars show the 95% confidence interval.

Distributional overlap ranged from 0.0 to 44.4% (Table 2). The distribution of *P. polionotus* did not overlap with any other predicted distributions. An overlapping area between lineages 1 and 2 existed at the eastern edge of the Rocky Mountains that made up 6.7% of the total predicted area (Table 1, Fig. 5A). Distributions of lineages 1 and 6 overlapped in California (15.5%; Table 2, Fig. 5B). Overlap between lineages 2 and 4 occurred in the central prairie region of the United States (5.6%; Table 2, Fig. 5C). Lineages 3 and 4 had the highest overlap (44.4%) of their predicted distributions, encompassing much of the northeastern United States (Table 2, Fig. 5D).

Table 1. Estimated correlation coefficients between genetic and environmental distance comparing sample locations of *P. maniculatus* lineages and *P. polionotus*, with geographic distance controlled using a partial Mantel test.

Genetic lineage	r	p-value
All combined	0.562	**0.001***
Lineage 1	0.078	0.258
Lineage 2	0.094	0.296
Lineage 3	0.412	**0.020***
Lineage 4	0.272	**0.004***
Lineage 6	–0.018	0.497
P. polionotus	–0.053	0.628

*significance at $\alpha = 0.05$.

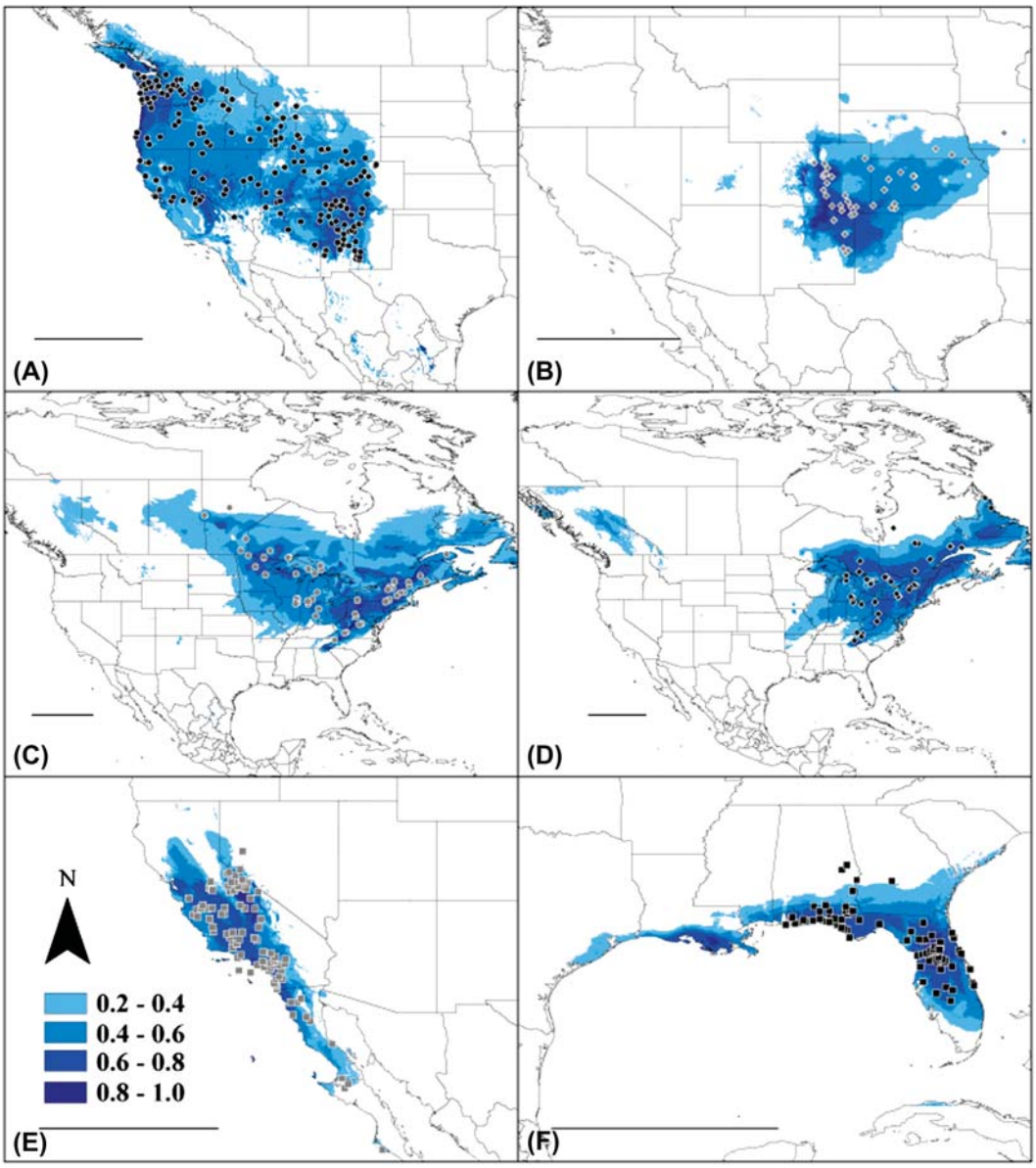

Figure 4. Predicted distribution using Maxent for (A) *P. maniculatus* lineage 1, (B) lineage 2, (C) lineage 3, (D) lineage 4, (E) lineage 6, and (F) *P. polionotus*. Lineages were determined based on phylogenetic analysis (Fig. 1). Distribution was determined based on 12 environmental variables. Shades indicate probability of occurrence, with darker shade being higher likelihood. Scale bars show 1000 km on each map.

Based on our estimates of niche overlap, we found *D* to range from 0.001 to 0.609, with lineage 2 and *P. polionotus* showing the largest differences while lineages 3 and 4 are the most similar (Table 2). All values are significantly different than predicted by randomly choosing location points for any of the pair-wise comparisons. Values of *I* showed the same pattern observed using *D* and ranged from 0.295 to 0.706 (Table 2). Niche overlap according to *I* indicated the lowest overlap between lineages 2 and *P. polionotus* and the highest overlap between lineages 3 and 4. All comparisons remained significant for both *D* and *I* when models were estimated from lower resolution environmental layers. This suggests that changing the resolution did not change our inferences of overlap in niche among lineages. Again, all comparisons show significantly lower values of overlap than expected by random processes (Table 2).

Discussion

As predicted, our phylogenetic analysis recovered distinct genetic lineages among members of the *Peromyscus maniculatus* species group. We also recovered distinct phylogeographic lineages within *P. maniculatus* similar to those found by other researchers (Lansman et al. 1983, Gering et al. 2009). These data add another example to the library of widely distributed species that show phylogeographic structure across North America (Hoffman and Blouin 2004, Pyron and Burbrink 2009). It should be noted that our phylogenetic inference relies on a single mitochondrial marker, which has its limitations. Increasingly, the fields of phylogenetics and phylogeography are utilizing multilocus approaches (Brito and Edwards 2009). Individual loci may not represent the evolutionary history of species as the locus itself is subject

Table 2. Percent geographic overlap in spatial niche space predicted by Maxent using minimum training presence criterion as thresholds for probability of occurrence of *P. polionotus* and 5 lineages of *P. maniculatus*. In addition, measures of niche overlap between genetic lineages of *P. maniculatus* and *P. polionotus*, using indices *D* and *I* proposed by Warren et al. (2008). Significance for the two indices determined by 100 pseudo-replicates in ENMtools, testing for significant difference in niche space than that expected by chance. Significance determined at $\alpha = 0.05$.

Lineage pair	Percent overlap	*D*	p-value	*I*	p-value
Lineage 1 vs lineage 2	6.7	0.149	< **0.001**	0.419	< **0.001**
Lineage 1 vs lineage 3	1.6	0.049	< **0.001**	0.355	< **0.001**
Lineage 1 vs lineage 4	4.7	0.052	< **0.001**	0.362	< **0.001**
Lineage 1 vs lineage 6	15.5	0.280	< **0.001**	0.533	< **0.001**
Lineage 1 vs *P. polionotus*	0.0	0.011	< **0.001**	0.308	< **0.001**
Lineage 2 vs lineage 3	5.6	0.109	< **0.001**	0.403	< **0.001**
Lineage 2 vs lineage 4	0.9	0.042	< **0.001**	0.343	< **0.001**
Lineage 2 vs lineage 6	0.0	0.015	< **0.001**	0.309	< **0.001**
Lineage 2 vs *P. polionotus*	0.0	0.001	< **0.001**	0.295	< **0.001**
Lineage 3 vs lineage 4	44.4	0.609	< **0.001**	0.706	< **0.001**
Lineage 3 vs lineage 6	0.0	0.006	< **0.001**	0.303	< **0.001**
Lineage 3 vs *P. polionotus*	0.0	0.012	< **0.001**	0.321	< **0.001**
Lineage 4 vs lineage 6	0.0	0.012	< **0.001**	0.307	< **0.001**
Lineage 4 vs *P. polionotus*	1.1	0.024	< **0.001**	0.333	< **0.001**
Lineage 6 vs *P. polionotus*	0.0	0.007	< **0.001**	0.304	< **0.001**

to evolutionary forces beyond those affecting the lineage. However, the majority of diversification events within the *P. maniculatus* species group occurred during the Pleistocene (Zheng et al. 2003, Van Zant and Wooten 2007), and adding nuclear gene sequences might not necessarily provide additional information (Martins et al. 2009).

The climatic analysis and ENM results for *P. maniculatus* and *P. polionotus* show that genetic divergence is correlated with niche divergence. Almost all lineages occupy significantly different climatic niches based on multiple lines of evidence. Our two multivariate statistical approaches, MANOVA and DA, support our interpretation that the phylogenetic lineages occupy significantly different climatic envelopes (Fig. 3). These two methods both utilize an a priori assumption of grouping data (i.e. phylogenetic lineages); however, we came to the same conclusion when using a principal component analysis (results not shown), which have no a priori requirement. The MANOVA and DA analyses are limited to known sample locations; however, by incorporating ENM we estimated the climatic and geographic distribution beyond the sample locations, and found lineages to be associated with significantly different climatic niches (Fig. 4, Table 2). The differences we observed in the climatic niches are not an artifact of geographic distance, as our partial Mantel test showed a positive correlation between genetic and environmental distance when we controlled for geographic distance (Table 1).

By evaluating the niche relationship between sister taxa we gained insight into how the environment may influence divergence events. Sister lineages 3 and 4 occupy very similar environmental spaces based on location data, which suggests these lineages have followed the pattern of niche conservatism (Fig. 3). If sister taxa are in allopatry, niche conservatism can reflect a pattern where lineages fail to adapt to the landscape matrix separating populations (Wiens and Graham 2005). Interestingly, the two lineages occupying northeast North America are parapatric based on haplotype distributions (Fig. 2), or sympatric based on the level of spatial

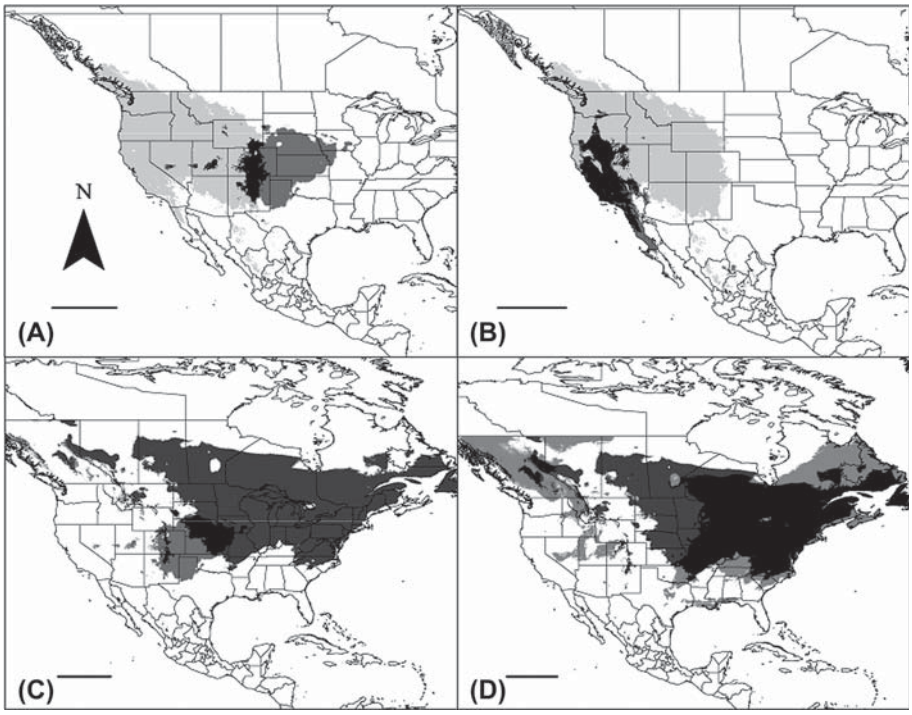

Figure 5. Overlap between predicted distributions of lineages using minimum training presence criterion as threshold of presence of suitable niche space. Grey areas are predicted allopatric while black areas are predicted overlap. (A) Predicted distribution of overlap between lineages 1 (light) and 2 (dark), (B) lineages 1 (light) and 6 (dark), (C) lineages 2 (light) and 3 (dark), and (D) lineages 3 (dark) and 4 (light). Scale bars show 1000 km on each map.

overlap (Table 2, Fig. 5D). In either case, due to the extensive spatial overlap found in their estimated distributions, current geographic isolation does not account for the genetic divergence between these two lineages. However, historical spatial isolation could have caused the patterns we observe as the two lineages are currently distributed in an area that was under ice during the last glaciations. Thus, the current pattern could be the result of secondary contact between populations expanding from glacial refugia, a pattern replicated in multiple taxa within this region of North America (Hoffman and Blouin 2004, Rowe et al. 2004). Another line of evidence suggests that niches may not be conserved between these two eastern lineages. Utilizing the methods of Warren et al. (2008), we found that these two lineages exhibit the highest level of niche similarity of any pair-wise comparison of genetic lineages, although they are still less similar to each other than expected by chance (Table 2). Lineages 3 and 4 were the only lineages showing a positive correlation between genetic and environmental distance. This suggests that genetic differentiation within these lineages may follow a climatic gradient across their distribution.

Sister lineages 1 and 2 from western North America support the hypothesis of niche divergence. They occupy significantly different climatic niches (Fig. 3, Table 2), with parapatric distributions (Fig. 2 and 5A). The overlap forms a potential contact zone between the two sister lineages, and this corresponds to the same area Osgood (1909) proposed to be a zone of integration between two subspecies. These lineages represent two ecomorphs (Blair 1950), with lineage 1 representing a forest type and lineage 2 a grassland type. These ecomorphs have distinct morphological differences, with the forest type having a long tail, large ears and large feet, while the grassland type is distinct with a short tail, small ears and small feet (Blair 1950). Selective pressure on niche may have influenced this lineage divergence, causing shifts in climatic niche and morphological differences between the two lineages. We cannot refute the possibility that the two lineages are connected by gene flow, which could explain the mixed nodal support for lineage 2 (PP = 0.65; BS = 70). Use of fast evolving nuclear markers such as microsatellites could aid in understanding the current interaction between these two lineages.

We determined that *P. polionotus* has diverged from *P. maniculatus* both genetically and ecologically (Fig. 1, 3 and 4). *Peromyscus polionotus* is hypothesized to have originated from a peripheral population of *P. maniculatus*, in particular from a lineage of short-tailed grassland mice (Blair 1950, Carleton 1989). However, Avise et al. (1983) found that *P. polionotus* clustered with a forest-dwelling form of *P. maniculatus*. Our phylogenetic analysis found low support for a sister relationship between *P. polionotus* and an ancestral *P. maniculatus* lineage (Fig. 1); the ancestral lineage diverged into both grassland (lineage 2) and forest-dwelling (lineage 1) forms (Fig. 1). While the evolutionary relationships of *P. polionotus* remain unclear, our data suggest that this species occupies a distinct niche compared to its closest relatives (Fig. 3). Our distribution models show some overlap among all geographically neighboring lineages for *P. maniculatus*, but overlapping with *P. polionotus* is limited to 1.1% overlap with lineage 4 (Table 2). This fits the expected distributions for the two species (Fig. 2), but it also suggests that the environmental space between *P. polionotus* and neighboring *P. maniculatus* lineages may have suboptimal conditions and could function as a barrier between the two species.

We also found possible contact zones in our ENM between non-sister lineages of interest. These areas match with proposed contact zones in the western United States between lineages 1 and 6, and in central regions, between lineages 2 and 3 (Fig. 5B, C; Osgood 1909, Hall 1981). Our data show that lineages 1 and 6 occupy significantly different environmental spaces, and our phylogenetic analyses shows they are isolated based on mtDNA. However, it is uncertain if hybridization may occur, or if a factor collinear with our climatic variables separate these two lineages in the contact zone. The contact zone between lineages 2 and 3 could represent another interaction between the long-tailed forest type (lineage 3), and the short-tailed grassland type (lineage 2). Field experiments in parts of *P. maniculatus*' distribution have shown that the two ecomorphs preferentially occupy grassland or forest (Hooper 1942, Wecker 1963), which suggests that these ecomorphs can be isolated by vegetative habitat preference. The divergence in the climatic niche we observe may reflect niche divergence between lineages 2 and 3 because of collinearity with patterns of vegetation separating the different ecomorphs.

Peromyscus polionotus and the lineages within *P. maniculatus* occupy unique environmental space, which could indicate that natural selection is a factor in lineage divergence, formation, and maintenance of species in a heterogeneous landscape (Rissler and Apodaca 2007). If the landscape had an impact in shaping the current diversity we observe within this group, such divergence would have had to occur recently, as the *P. maniculatus* species group is a recent radiation with divergence occurring in the Pleistocene (Zheng et al. 2003, Van Zant and Wooten 2007). This is possible as studies have found that adaptation to new environmental conditions can occur over short periods of time, with the niche being labile over time (Evans et al. 2009, Dormann et al. 2010). However, to validate niche as a driving factor in lineage divergence, phenotypic traits must be identified related to adaptations for persisting in their niche and resulting in reproductive isolation between lineages (Graham et al. 2004, Kozak et al. 2008). We do not identify such traits attributed to lineage divergence in our study, but two sister lineages (lineages 1 and 2) and two non-sister lineages (lineages 2 and 3) may represent morphologically different groups: forest and grassland ecomorphs. These morphological differences between lineages 1 and 2 could possibly show differential adaptation to different environments, whereas adaptation to different niche space between lineages 2 and 3 may serve to avoid hybridization between these closely related lineages.

Conclusions

A body of evidence is accumulating that niche divergence is common and can occur over short periods of time (Evans et al. 2009, Dormann et al. 2010). Even within closely related species, evidence for niche conservatism and niche divergence is observed (Evans et al. 2009, McNyset 2009, Pyron and Burbrink 2009). This pattern is evident for *P. maniculatus*

and *P. polionotus*, in which we demonstrate niche divergence and niche conservatism in different lineages. Our study suggests a potential for climate, or a collinear variable, to be a relevant component in the diversification of widely distributed taxa. Many taxa inhabit wide distributions and encounter heterogeneous landscapes. This heterogeneity of habitat is often reflected in phylogeographic patterns similar to those we observed (Hoffman and Blouin 2004, Fontanella et al. 2008). The results of this study suggest that other wide-ranging taxa may also exhibit a mosaic of niche divergence and niche conservatism.

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Supplementary material (Appendix E6994 at <www.oikosoffice.lu.se/appendix>). Appendix 1.