

APPLICATION

Fast mvSLOUCH: Multivariate Ornstein–Uhlenbeck models of trait evolution on large phylogenies

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Abstract

1. The **PCMBase** R package is a powerful computational tool that enables efficient calculations of likelihoods for a wide range of phylogenetic Gaussian models.
2. Taking advantage of it, we redesigned the R package **mvSLOUCH**.
3. Here, we demonstrate how the new version of the package can be used to thoroughly examine the evolution and adaptation of traits in a large dataset of 1252 vascular plants through the use of multivariate Ornstein–Uhlenbeck processes.
4. The results of our analysis demonstrate the ability of the modelling framework to distinguish between various alternative hypotheses regarding the evolution of functional traits in angiosperms.

KEYWORDS

maximum likelihood inference, multivariate Ornstein–Uhlenbeck process, multivariate phylogenetic comparative methods, **mvSLOUCH**, R

1 | INTRODUCTION

Most studies that utilize phylogenetic comparative methods (PCMs) focus on single traits. However, evolution is inherently a multivariate phenomenon. Traits are seldom genetically independent; for example, one trait's evolution may be influenced by

selection on another trait (Lande, 1979; Lande & Arnold, 1983; Walsh & Blows, 2009). Moreover, traits may undergo correlational selection (Svensson et al., 2021). Given this complexity, univariate models are insufficient for fully understanding the implications of the multivariate nature of selection and evolution. Therefore, multivariate models are essential to more accurately represent the

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evolution of traits. The Ornstein–Uhlenbeck (OU, Equation (1)) process is particularly effective in this regard. Its main benefit is modelling how a trait's optimum—defined by the $\mathcal{A}(t)$ parameter—adapts in response to varying conditions. This model facilitates the testing of various evolutionary hypotheses, such as those concerning trade-offs (Bartoszek et al., 2012) and modularity (Bartoszek et al., 2023). Here, we present the updated version of **mvSLOUCH** software that allows users to quickly fit multivariate OU models to large datasets, address measurement errors and missing data, and precisely define interacting traits and the direction of these interactions.

We define the OU process (see Appendix SA2 for additional details and interpretations) for a k -dimensional trait, $\mathbf{x}(t)$, as

$$d\mathbf{x}(t) = -\mathbf{A}(\mathbf{x}(t) - \mathbf{0}) dt + \Sigma_y d\mathbf{W}(t) \quad (1)$$

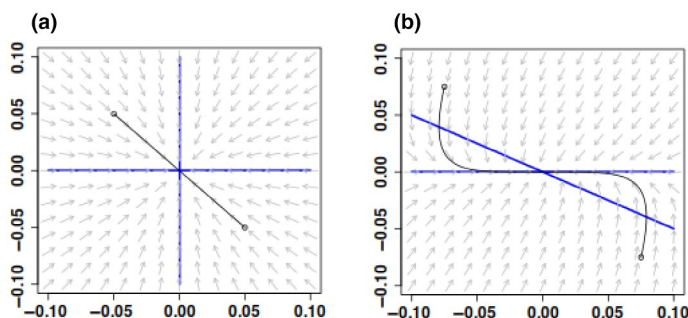
where $\mathbf{W}(t)$ is a k -dimensional standard Brownian motion. Equation (1) models the changes in a suite of k traits over an infinitesimal amount of time. The parameter $\mathbf{A}$ is the matrix counterpart of the selection strength parameter (usually denoted α ; e.g., Butler & King, 2004). It is crucial to underline that $\mathbf{A}$'s entries are difficult to interpret directly—one should rather investigate its eigenvalues and eigenvectors (Figures 1 and S.1). It is important to point out that from **mvSLOUCH** 2.0.0 onwards, users can not only fit models allowing values to populate different parts of the $\mathbf{A}$ and Σ_y matrices (e.g., 'Diagonal', 'UpperTri', 'LowerTri', 'Symmetric'), but can also customize individual cells, allowing

the user to test several highly specific sets of trait interactions upon real data and compare their fit (see Section 3).

Many software packages developed in the last two decades for estimating Equation (1)'s parameters faced issues with long computation times. Computational running time limited analyses to ca 400 species for up to six traits. Initially, simplifying assumptions reducing the number of parameters to be estimated were made. For example, the first multivariate OU-based method (**ouch**, Butler & King, 2004) was restricted to a symmetric-positive-definite drift matrix. Bartoszek et al. (2012) introduced the **mvSLOUCH** (Multivariate Stochastic Linear Ornstein–Uhlenbeck Models for Phylogenetic Comparative Hypotheses) R (R Core Team, 2019) package that can handle and compare a wide variety of parametrizations for hypotheses concerning multivariate trait evolution.

From a broader perspective, the observations at the tips of a phylogeny come from a multivariate [K -dimensional, with no missing observations K is number of tips (n) times number of traits (k)] normal distribution with a covariance matrix, $\mathbf{V}$, that depends on the tree and evolutionary process. One can obtain the likelihood directly using a standard multivariate normal density function. However, this comes at a computational cost—obtaining $\mathbf{V}$ requires on the order of K^2 calculations, and then one needs to find its determinant and inverse. Constructing $\mathbf{V}$ is therefore a major computational bottleneck.

Several approaches have been proposed to provide faster likelihood calculation algorithms. In particular, Mitov et al. (2020) considered a wide family of Gaussian models, the $\mathcal{C}_{Linv}$ family (Def. S.1)



Panel A

(a) Independent attraction, diagonal $\mathbf{A}$ with positive elements

$$\begin{array}{cc} & \begin{array}{cc} \text{Trait 1} & \text{Trait 2} \end{array} \\ \begin{array}{c} \text{Trait 1} \\ \text{Trait 2} \end{array} & \begin{bmatrix} 0.01 & 0 \\ 0 & 0.01 \end{bmatrix} \end{array}$$

(b) coadaptation mediated by trait 2 upper-triangular $\mathbf{A}$

$$\begin{array}{cc} & \begin{array}{cc} \text{Trait 1} & \text{Trait 2} \end{array} \\ \begin{array}{c} \text{Trait 1} \\ \text{Trait 2} \end{array} & \begin{bmatrix} 0.01 & 0.02 \\ 0 & 0.05 \end{bmatrix} \end{array}$$

FIGURE 1 Illustration of independent adaptation and coadaptation. The phase portraits, based on $\mathbf{A}$, reflect the dynamics of the mean value of the OU process, where the optimum ($\mathcal{A}$) for both traits is 0. Trait 1 is presented on the abscissa, and Trait 2 on the ordinate. In the plot, the influence of stochastic perturbations captured by Σ_y is omitted (for a thorough graphical depiction of diffusion parameterizations, see Clavel et al., 2015). Two example starting points (black open dots) and their subsequent trajectories (indicated by the black lines emerging from the dots) are shown moving towards 0. The grey arrows indicate the direction of evolution, and the blue lines represent the nullclines. We only illustrate the most general scenarios: (a) where the traits do not influence each other's average trajectory (diagonal $\mathbf{A}$) and (b) where one trait's path is mediated by the other (upper-triangular $\mathbf{A}$). In the Supplementary Material (Figure S.1; Table S.1), we show more scenarios for $\mathbf{A}$, including both-way interactions (non-triangular $\mathbf{A}$) and repulsion ($\mathbf{A}$ has negative eigenvalues). Interpretations of the paths towards the optimum (notice that the optimum is $\mathcal{A}$, so neither the traits nor $\mathbf{A}$ affect the optimum itself, $\mathbf{A}$ only affects the shape of the path) come from the investigation of $\mathbf{A}$'s eigenvalues and eigenvectors. In (a), we can see that both traits move directly towards the optimum in a symmetric fashion (as both have equal eigenvalues equalling 0.01 and $(1, 0)$, $(0, 1)$ are the eigenvectors), while in (b), the path is more nuanced. There is an initial pull in one direction (eigenvector $(0.5, 1)$ corresponding to an eigenvalue of 0.05), and after the 'y' trait has approached 0, we observe a strong change in the direction of the pull (eigenvector $(1, 0)$ corresponding to an eigenvalue of 0.01). Note that we never observe a straight line of the pull—rather, it is a curve as the direction of movement is a (non-linearly) weighted average of the two eigenvectors, with speeds of movement defined by the two eigenvalues.

on phylogenies. Common uni/multivariate PCM models like the BM, BM with drift, OU, punctuated equilibrium (OU with jumps at speciation), white noise, early burst/accelerating-decelerating (Blomberg et al., 2003; Harmon et al., 2010) or BM with a trend (Harmon et al., 2010) all belong to this family. The likelihood under these models can be found in the procedures implemented by Mitov et al. (2020) in the **PCMBase** (with its C++ backend, **PCMBaseC++**) package. **PCMBase** serves as a computational engine for calculating the likelihood for C_{Linv} models of evolution. From **mvSLOUCH 2.0.0** the likelihood is calculated using **PCMBase**. The switch from the algorithm requiring **V** explicitly, to Mitov et al.'s (2020) one opens completely new modelling and estimation possibilities for **mvSLOUCH**. Much larger trees can be considered and complex simulation studies undertaken. It is now possible to investigate the effects of measurement error, effects of multiplying the data by a matrix (e.g., by an orthogonal matrix, i.e., pre-rotation), the likelihood surface, or applicability of various information criteria/model selection techniques.

mvSLOUCH and **PCMFit** (Mitov et al., 2019), handle the widest class of OU-based models among all PCM software known to us (Table S.2). Both packages, based on **PCMBase**, seamlessly handle polytomies, missing data, and non-ultrametric phylogenies. Fossil data can be incorporated by placing measurements on short, non-contemporary tip branches in line with their hypothesized timing. **PCMFit** is able, in principle, to handle every model that **mvSLOUCH** handles. While **PCMFit** is able to handle multiple models on the phylogeny (e.g., mixing BM and OUs models) and estimate where models switched, **mvSLOUCH** is dedicated towards OU-type models. It allows for direct constraining of parameters so that they correspond to specific evolutionary hypotheses (potentially non-trivial to implement in **PCMFit**) and returns summary statistics describing the relationships between the traits from multiple, biologically relevant, perspectives. In particular, **mvSLOUCH** returns half-lives in terms of tree height, evolutionary and optimal regressions, and correlations between all pairs of traits. Parametrizations of **A** based on its eigendecomposition, and control over individual entries, are directly offered by **mvSLOUCH**. This increase in **mvSLOUCH**'s inference capability provides new possibilities for users. Here, we describe how to take advantage of this additional functionality by means of an example analysis of functional traits in angiosperms using a large phylogeny. We provide extensive details on the probabilistic framework behind **mvSLOUCH** (Supplementary Material, especially the multivariate OU process), describe newly implemented functions (e.g., building blocks for a phylogenetic regression study, Appendix SB3), present analytical estimation formulae for the BM case, discuss initial seeds for numerical estimation procedures (Appendix SE), and demonstrate the magnitude of the speed-up due to using **PCMBase** (Appendix SG).

2 | NEW FEATURES OF mvSLOUCH

We present functionality that offers the user a sophisticated toolbox for studying OU-based evolution. For technical details, exemplary

code, and analyses, we refer to the Supplementary Material, the package's documentation and vignette, and the accompanying GitHub repository (https://github.com/krzbar/KJVJMRKK_mvSLOUCH). The user can specify detailed evolutionary hypotheses through classes of the OU process's matrix parameters (see Appendix SA3; Bartoszek et al.'s (2023) Supplementary Material, Appendix SB) or by controlling (e.g., forcing to 0) individual entries of these matrices. Then, model selection can be performed on sets of competing hypotheses. **mvSLOUCH**'s *estimate.evolutionary.model()* function (developed by Xiao et al., 2018) can compare multiple models and return the best one (under Akaike's information criterion corrected for sample size, AIC_c (Hurvich & Tsai, 1989); in Bartoszek et al. (2023), we also explored AIC_c 's model selection capabilities; however **mvSLOUCH** also returns other information criteria). This function provides an intuitive explanation concerning the best model. A function to compute parametric bootstrap confidence intervals (CIs) was implemented approximately 10 years ago, but only now it is possible to obtain appropriate sample sizes (see Section 3). The fast likelihood evaluation method allows for efficient calculation of quadratic forms with respect to **V** (Appendix SB3), allowing for the evaluation of phylogenetic regressions (Appendix SB4) and, consequently, analytical generalized-least-squares estimators for BM models (Appendix SD). **mvSLOUCH** now offers the possibility to perform custom phylogenetic regressions and to calculate the phylogenetic residual sum of squares for an arbitrary vector (Appendix SB3). These give the user the possibility to regress on arbitrary predictors, not necessarily phylogenetically related ones, and can be building blocks for methods based on quadratic forms. New features have been added to the package, including the ability to estimate the root state under OU models (previously—fixed at the primary optimum). For OU models, estimation is carried out from multiple starting points, one derived from analytical considerations (see Section Appendix SE). The user can extend optimization from a good starting point from previous analyses (see Section 4) and also has control over the number of iterations of the numerical optimizer, which can be adjusted to reduce run-time. The package's output has been extended with various R^2 values (Appendix SB2).

3 | EXAMPLE ANALYSIS: EVOLUTION OF FUNCTIONAL TRAITS IN A DATASET OF 1252 VASCULAR PLANTS

Size determines many morphological, anatomical, and physiological characteristics of living organisms. However, the scaling of these various traits with size (allometries) reveals considerable variation, likely generated by natural selection in response to environmental problems (Voje, 2016). Although the general allometric relationships between plant size and their leaves, roots, fruits, and seeds have been documented (Niklas, 1994), there are several interesting exceptions. One of them is the relationship between seed mass and leaf area, where a wide range of leaf area values co-occur with small seed mass values. Yet, this leaf area variation narrows as seed mass increases,

forming a triangle-shaped distribution (Cornelissen, 1999; Santini et al., 2017). We intuitively expect a positive scaling relationship, because of the observed positive relationships between leaf area and twig thickness as well as between leaf area and size of infructescence. The absence of this correlation, at least in tested woody and annual plants, was due to the occurrence of species having large leaves and small seeds (Cornelissen, 1999; Santini et al., 2017). It was hypothesized that differences among habitats in plant ecological strategies play a critical role. For example, while stress-tolerant and competitive strategists possess either small seeds and leaves or large leaves and seeds respectively, there is a group of ruderal plants with small seeds and large leaves occupying early successional habitats (Cornelissen, 1999; Santini et al., 2017).

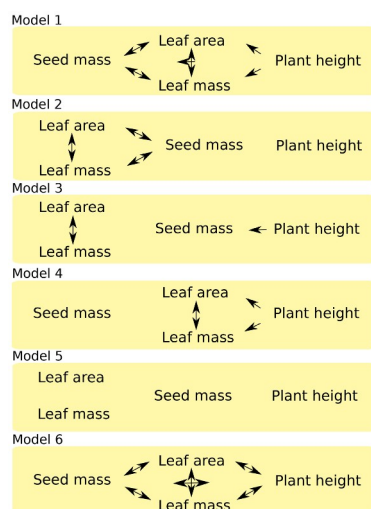
mvSLOUCH provides an opportunity to test these allometric hypotheses in a comparative framework consistent with the process of natural selection. Especially important is the option to fit parameters of multivariate OU processes, Equation (1), (OUOU models—some traits are interpreted as predictors and others as responses). This allows both independent and dependent continuous variables to reflect adaptive evolution. A second advantage of **mvSLOUCH** is that its multivariate nature allows for the construction of relatively sophisticated evolutionary models, that can be interpreted in some causal framework (Reitan et al., 2012).

We obtained measures of leaf mass, leaf area and seed mass for 1252 European angiosperm species from Carmona et al. (2021), who curated these measures from the TRY database (Kattge et al., 2011). To this, we added a fourth trait, plant height, which we curated from TRY. We collected Ellenberg indicator values for nitrogen (ELN) (from Chytrý et al., 2018), which are divided into nine classes (1–9) ranging from low (1) to high (9) productivity habitats. We mapped

the nine ELLN categories to our phylogeny using parsimony with delayed transitions (DELTRAN) using **mvSLOUCH::fitch.mvsl()** (Table S.6; Figure S.6). There are 19 branches which required manual resolution, based on the fact that Ellenberg values are ordinal. The assignment is visible in the supplementary material's code. It should be pointed out that there are more sophisticated approaches, like stochastic character mapping (e.g., *phytools::simmap()*; Huelsenbeck et al., 2003; Nielsen, 2002; Revell, 2012), that we encourage users to explore. In Appendix SC7, we provide a small example of how to integrate *phytools::simmap()* output with **mvSLOUCH**. Furthermore, using probabilistic approaches would provide estimates of uncertainty for regime placements. The dated phylogeny is the supertree of plants used by Carmona et al. (2021).

We tested several adaptive hypotheses concerning the effect of habitat productivity on allometric relationships by altering **A**. The **A** matrices were parametrized in six ways (visualized in Figure 2) as follows:

1. Plant height is the driving factor of changes in leaf and seed traits, which in turn affect each other's optima; plant height, which is linked to the species' competitive ability, may be affected by productivity, with taller plants selected in more N-rich habitats.
2. This model differs from the previous one in that plant height does not affect leaf and seed traits, which evolve independently; this model assumes that the diversity of plant architectures (branching pattern, growth form, etc.) in angiosperms is very high, preventing any simple allometric relationship between height, leaf and seed traits. We expect a stronger effect of habitat regimes on leaf and seed traits, yet less so on plant height.



Model		A				Model		A			
		PH	SM	LA	LM			PH	SM	LA	LM
1	PH	+	0	0	0	2	PH	+	0	0	0
	SM	?	+	?	?		SM	0	+	?	?
	LA	?	?	+	?		LA	0	?	+	?
	LM	?	?	?	+		LM	0	?	?	+
3	PH	+	0	0	0	4	PH	+	0	0	0
	SM	?	+	0	0		SM	0	+	0	0
	LA	0	0	+	?		LA	?	0	+	?
	LM	0	0	?	+		LM	?	0	?	+
5	PH	+	0	0	0	6	PH	?	?	?	?
	SM	0	+	0	0		SM	?	?	?	?
	LA	0	0	+	0		LA	?	?	?	?
	LM	0	0	0	+		LM	?	?	?	?

FIGURE 2 Left: Graphical representation of models 1–6. Arrows indicate which trait affects which trait through **A**. Right: Classes of **A** corresponding to each of the six models considered in the analyses of the vascular plants' functional traits data. 0 means the specific entry is constrained to be 0 in the estimation procedure, and +, that it is constrained to be positive. Entries denoted by ? are free to vary over the whole real line, they are not constrained to be all equal to each other in the matrix. These are, respectively, represented by 0, '+' and NA when calling **mvSLOUCH**. The order of the variables for the **A** matrices is plant height (PH), seed mass (SM), leaf area (LA), and leaf mass (LM).

3. We remove adaptive links between leaf and seed traits here; we assume that taller plants and bigger seeds are advantageous in nutrient-rich, competitive habitats.
4. Contrary to model 3, we assume independent evolution of seed mass while considering adaptive links between plant height and leaf size; nitrogen-rich habitats may select for tall competitive plants with large leaf areas capturing more energy.
5. Here there are no adaptive links among studied traits; setting **A** to a diagonal matrix means that all traits are independently shaped by selection. This could be a plausible assumption given angiosperms are hugely diverse and display a myriad of life history strategies.
6. In this most parameter-rich model, we allow the $\mathbf{A}_{\text{Eq}(1)}$ matrix to be fully parametrized, which we interpret as a trait-trait adaptive co-evolution as expected from allometric theory.

These six models were contrasted with the BM model. We point out that it is possible to run all of these analyses in a single function call, `mvSLOUCH::estimate.evolutionarymodel()`. However, given the tree's size and number of initial seeds, the running times would be very large, risking the loss of all calculations in case of, for example, a computer's crash. We conducted two sets of analyses for each of these models, in order to explore differences in the non-adaptive component of the trait evolution. In the first set, Σ_{yy} was diagonal, implying no interactions between the traits in the noise component of evolution. In the second set, Σ_{yy} was upper-triangular, indicating interacting evolution of the traits due to, for example, developmental constraints or covariation with other unmeasured trait under selection. To improve maximum likelihood (ML) estimation, 500 runs of each model were conducted from the same 500 starting seeds for the optimizer. Five hundred unique starting points were used for each of the Σ_{yy} diagonal and upper-triangular versions (see 'Numerical optimization' section of **mvSLOUCH**'s vignette for an analogous example). All analyses were completed using the computing cluster FUN-K at the Biological and Chemical Research Center, University of Warsaw using one node with 48 threads. It took 6.3 days to accomplish all computations, including bootstrapping.

4 | RESULTS AND DISCUSSION

After fitting all six models, we found that the model with the lowest AIC_c was model 6 with Σ_{yy} diagonal. However, the alternative model 6 (Σ_{yy} upper-triangular) had a lower log-likelihood value than model 6 with Σ_{yy} diagonal. This cannot happen if the optimizer hit the global optimum, as a diagonal Σ_{yy} is a special case of a Σ_{yy} upper-triangular. We therefore used the model 6 Σ_{yy} diagonal output as a starting point for a new run of model 6 with Σ_{yy} upper-triangular (using `ouchModel()`'s `start_point_for_optim` argument), from which we then obtained our lowest, among all models, AIC_c score. This highlights that, after fitting several models to a dataset, it is beneficial to take sub-models with good likelihood and AIC_c scores,

and use these as starting points for their generalizations. In fact, we followed this procedure for all models, refining the best found diagonal Σ_{yy} point to Σ_{yy} upper-triangular. This refinement usually found a point with a higher likelihood, than when started with a random numerical seed. Interestingly, in a number of cases, the diagonal structure of Σ_{yy} was retained in the ML point, despite the optimization algorithm being free to vary the off-diagonals. We point out that many repetitions of the same model, using different random seeds/starting points, are necessary to obtain the best possible estimates of model parameters. As the likelihood surface of phylogenetic OU models is still not well understood, we cannot yet guarantee a global optimum. To gain from using the output of one estimation as the input for another, the parametrization of the latter should be less restrictive (Σ_{yy} upper-triangular is the most general one). In addition, after many repetitions (computational constraints allowing) it is advisable to check if the highest scoring subset results in similar parameter estimates; in particular, the optima, and compound statistics like regressions, covariances and correlations. The estimates of $\mathbf{A}_{yy}$, and Σ_{yy} can be very variable but still lead to similar covariance or regression estimates.

After the reanalysis, model 6 with Σ_{yy} upper-triangular had the lowest AIC_c value (16,381.058, Table 1). Because the second best model (model 6 with Σ_{yy} diagonal) had AIC_c greater by over 46 units from the best one, there is little empirical support for any model but model 6 with Σ_{yy} upper-triangular. Model 6 is superior to all other models, as the next best model (Model 3) is over 1774 AIC_c units away, regardless of the form of Σ_{yy} .

It is noteworthy that there is a high variation in the end values of the likelihood function among different runs of the same model. For the best model, these varied in the range $-172,526.72$ to -8127.739 . Additional outputs for the lowest AIC_c fit for each of the six main models are provided in the supplement, including values of the estimated **A** (Table S.8) and Σ_{yy} (Table S.9) matrices, and primary optima vectors (Tables S.10–S.15).

The best model is consistent with an adaptive effect of habitat productivity on all organ traits. We can observe a positive shift in trait optima values as the ELL-N increases (Figure 3; Table S.15). A possible explanation for this pattern is construction cost, which can limit trait size in nutrient-poor habitats. This has been demonstrated in many studies, especially for leaf size, which decreases at the inter- and intraspecific level (e.g. Givnish, 1987; Xu et al., 2009). This agrees with our analyses as leaf size, represented here by mass and area, showed the most substantial shift across the gradient of habitat productivity. Because our model assumes trait-trait co-evolution of all four studied characters, the primary optimum for each trait is influenced not only by habitat productivity, but also by all other traits. The strength of this influence comes from cross-trait effects in the primary optima, summarized by **A**, as well as from non-adaptive interacting evolution summarized by Σ_{yy} . This influence is not identical in all cases as revealed by regression coefficients (Table S.16).

Plant size, the leading contributor to competition for light and propagule dispersal is most affected by leaf mass and weakly by

Model	Σ_{yy} diagonal				Σ_{yy} uppertriangular			
	LogLik	AIC _c	R ²	df	LogLik	AIC _c	R ²	df
1	-10,598.94	21,305.04	0.052	53	-10,607.68	21,334.80	0.05	59
2	-9968.793	20,038.615	0.076	50	-9747.051	19,607.392	0.054	56
3	-9996.868	20,088.645	0.077	47	-9024.085	18,155.326	0.035	53
4	-10,373.82	20,844.58	0.082	48	-9918.165	19,945.529	0.105	54
5	-9995.955	20,080.709	0.088	44	-9533.795	19,168.618	0.040	50
6	-8156.907	16,427.104	0.088	56	-8127.739	16,381.058	0.094	62
BM	—	—	—	—	-10,912.55	21,853.19	0.003	14

Note: The best model discussed is marked in bold.

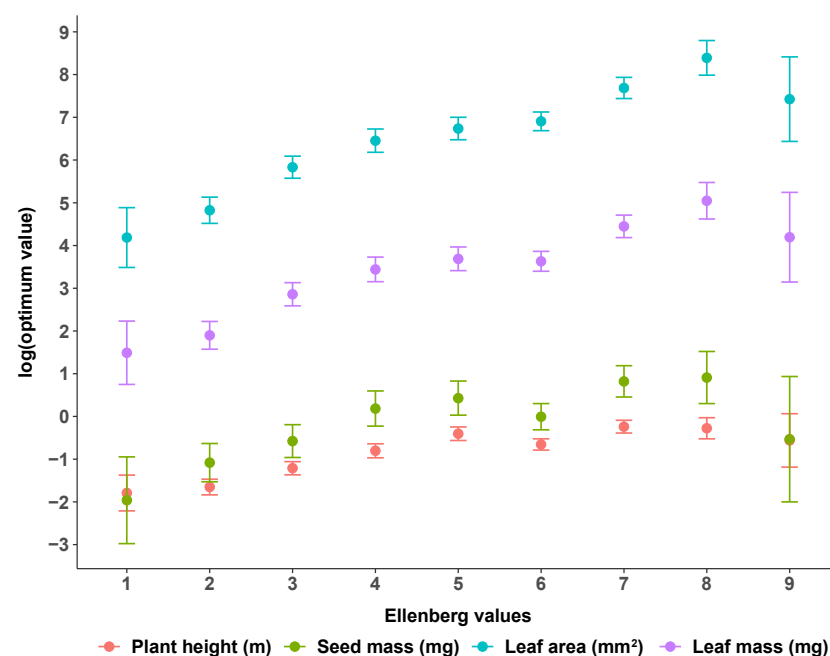


TABLE 1 The best AIC_c scores, from the 2001 fits per model, along with the log-likelihood (LogLik), R² and degrees of freedom (df) values for the six models analysed with diagonal and upper-triangular Σ_{yy} .

FIGURE 3 The deterministic optimum values for four morphological traits (from the best-fitting model). These values display a general trend of increasing character values with habitat productivity represented here by ELL- N. The plotted points indicate the estimated values and the whiskers represent the 95% regression-based confidence intervals (whiskers). See Table S.15 for the numerical values with CIs.

TABLE 2 Phylogenetic half-lives with 95% parametric bootstrap (500 bootstrap replicates) CI reported here as percentage of tree height in the eigenvector directions for the best model 6.

Model 6	Directions (eigenvectors)			
	e_1	e_2	e_3	e_4
PH	0.007	0.015	-0.686	0.184
SM	-0.046	0.351	-0.353	0.863
LA	0.122	-0.668	-0.367	0.351
LM	0.991	-0.656	-0.52	0.313
Half-life	0.0002%	0.033%	0.407%	2.028%
(CI no seed)	(0.107%, 72.392%)	(7.416%, 80.331%)	(26.214%, 192.568%)	(66.732%, 1630.682%)
(CI seed)	(0.00015%, 0.00033%)	(0.0262%, 0.065%)	(0.333%, 1.111%)	(0.489%, 2.613%)

Note: The abbreviations relate to measured plant height (PH), seed mass (SM), leaf area (LA), and leaf mass (LM). As bootstrapping involves running 500 simulations, followed by re-estimation (see Appendix SC6), there is no guarantee that CIs will encapsulate the estimated parameter values from the best model. The first row of the CIs corresponds to the situation where no numerical seed is provided for the likelihood optimization for the bootstrap replicates; the second row when the same numerical seed, as in the case of the best found estimate, is provided for the likelihood optimization. The CIs in the first row do not cover the true values, illustrating the known difficulties of estimating the $\mathbf{A}$ scalar parameter in the one dimensional case (Cressler et al., 2015).

other traits (Table S.17). This underscores leaf mass as the dominant factor driving the evolution of plant height. Seed mass was positively affected by both leaf area and plant height, but negatively by leaf mass. The unexpected negative relationship between seed and leaf mass (but notice that the CIs cover 0), might stem from the trait distribution on the phylogeny or from a preponderance of plants in the dataset that have heavy leaves and small seeds, such as pioneer species (Cornelissen, 1999). The positive correlation of seed mass with plant height can perhaps be attributed to the basic fact that small plants can bear only small seeds while larger plants can support much larger ones. Again, the bootstrap CIs cover 0 (when the numerical seed is not fixed). Interestingly, our results revealed that seed mass only has a marginal affect on other traits (regression coefficient in all cases does not exceed 0.11) in spite of being used as a predictor in other studies, such as those analysing leaf traits (Hodgson et al., 2017). This might suggest that directional selection can change seed mass without a strong effect on other traits. However, seed mass is significantly influenced by shifts in the values of other characters.

The last two studied traits, leaf mass, and leaf area, have a strong mutual effect on each other, as evidenced by the estimated regression coefficients of 0.910 and 0.846, respectively. The bootstrap CIs for these coefficients do not include 0 (Table S.16). The influence of other traits on leaf mass and leaf area is much weaker (Table S.17). Leaf area and mass have been shown to be strongly correlated with many physiological processes in plants, such as maximum photosynthetic rate, leaf life span or relative growth rate (Moles, 2018), and both are certainly optimized by selection to adjust a plant's physiology to a particular environment.

Our model also provides information about the tempo of adaptation, measured as a half-life (Bartoszek et al., 2012; Hansen, 1997). Here, unlike in univariate models, the phylogenetic half-lives can be interpreted as rates of adaptation towards the primary optimum along the directions in the trait space described by the corresponding eigenvectors. All four eigenvectors and eigenvalues in our model indicated adaptation to changing primary optima, ranging from 0.0002% to 2.028% of tree height, translating to ca. 300a–2.82Ma (Table 2). It is important to emphasize that these half-lives refer to the adaptation of a group of complex developmental traits, not just one trait as in univariate analyses. Given this, the rate of adaptation can be considered very rapid—a pace expected for ecologically relevant traits. For example, the first eigenvector, associated with the lowest half-life of 300a, is dominated by leaf mass (entry for this trait is 0.991, compared to the second largest entry of 0.122). Although this half-life might be underestimated, it aligns well with empirical studies. Leaf traits have been demonstrated to frequently experience strong selective pressure and possess high evolvability (Donovan et al., 2011). In consequence, they evolve rapidly within species or between closely related taxa inhabiting contrasting environments (Donovan et al., 2011).

Our **mvSLOUCH** analyses provided several interesting additions to existing studies. Our best model showed that habitat productivity positively influences the adaptive optima of all studied traits. In

addition to habitat quality, all traits co-evolve adaptively. However, the strength of the effect of each trait on the optimum of the other traits is not identical: plant height is influenced mainly by leaf mass, seed mass by all traits approximately equally, while leaf area and mass affect each other's optima with weaker influence of other traits. All adaptive changes are rapid on macroevolutionary time scales, taking no more than 2.82Ma. A key motivation of the above example was to demonstrate the feasibility of **mvSLOUCH** exploring several competing hypotheses of adaptation and evolution for large, multivariate datasets. Such a study was made possible due to the immensely reduced running times (Appendix SG) that allowed for thousands of estimation reruns under different setups and starting points. This is also facilitated by simplicity of coding of the different biological hypotheses—the user just needs to write a matrix with entries NA (anything), '+' (positive), '-' (negative) or '0' (equals 0, any other numerical values can be provided here).

Two drawbacks of our analyses have to be acknowledged. Firstly, we did not have measurement error estimates—only the mean value for each species. It has to be reminded that measurement error can have profound effects, resulting in biased estimates (Hansen & Bartoszek, 2012) or masking of adaptive effects (Bartoszek et al., 2023). Secondly, in order to focus on the possibilities of **mvSLOUCH** itself, we mapped the regimes using **mvSLOUCH**'s in-built Fitch algorithm (Fitch, 1971), using the simplest handling of ambiguous mapping, with additional post-hoc manual assignment of the remaining 19 unresolved branches. There are more advanced regime placement methods that we would encourage users to explore.

Lastly, the user needs to be aware of some limitations of **mvSLOUCH**. Running times can be still long for high-dimensional models and large trees. As observed in our study, the variability of the returned likelihood value is very high—and hence, also of the parameters' estimates. This can be mitigated, at the moment, only by considering multiple starting points for the optimizer. Finally, model identification capabilities are not entirely clear (Bartoszek et al., 2023) and some parameters are more difficult to estimate than others, especially **A**, under very general parameterizations (**mvSLOUCH** will warn if a known unreliable parameterization is chosen). However, these seem to be reservations common to all contemporary multivariate PCM software.

On the other hand, the improved likelihood calculation algorithms and, in turn, reduced running times, will allow for investigating these issues. We are able to design now large simulations studies (e.g. Bartoszek et al., 2023; Bartoszek, Fuentes-González, et al., 2024) that can aim to identify which model pairs (biological hypotheses) are distinguishable, which parameters can be consistently estimated, what tree sizes (under a given tree model) are required, and what are the effects of, for example, measurement error or missing data. Multivariate models are more complicated to fit to empirical data and require substantially more computational power than their univariate counterparts. However, the upside is the ability of these models to reveal more realistic knowledge of adaptation and evolution among suites of traits. Simpler models should not be preferred if the question at hand demands complex multivariate approaches.

AUTHOR CONTRIBUTIONS

Krzysztof Bartoszek, John Tredgett Clarke, Jesualdo Fuentes-González, Jason Pienaar, Marcin Piwczynski and Kjetil Lysne Voje conceived the ideas and designed methodology; John Tredgett Clarke, Marcin Piwczynski and Radosław Puchałka collected the data; Krzysztof Bartoszek, John Tredgett Clarke, Venelin Mitov and Kjetil Lysne Voje developed the software for the analyses; Krzysztof Bartoszek, John Tredgett Clarke, Marcin Piwczynski and Kjetil Lysne Voje analysed the data; Krzysztof Bartoszek led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication. Author ordering is alphabetical.

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflict of interest.

DATA AVAILABILITY STATEMENT

mvSLOUCH is available at <https://cran.r-project.org/web/packages/mvSLOUCH/> and <https://github.com/krzbar/mvSLOUCH>. The Zenodo <https://zenodo.org/records/11286569> (Bartoszek et al., 2024b) repository contain scripts, data, random seeds and simulation outputs used. These can also be found in the GitHub https://github.com/krzbar/KJVJM_RKK_mvSLOUCH repository, alongside multiple other analyses by **mvSLOUCH**. Data are available via the Dryad repository <https://doi.org/10.5061/dryad.8w9gk3vt> (Bartoszek et al., 2024a).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1: The probabilistic framework.

Appendix S2: The phylogenetic regression.

Appendix S3: The fast mvSLOUCH R package.

Appendix S4: Analytical estimation formulae for BM using the RSS.

Appendix S5: Initial seeds for numerical estimation procedures.

Appendix S6: Evolution of functional traits in vascular plants analysis.

Appendix S7: mvSLOUCH timings.

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