

Appendix from P. A. Hohenlohe and S. J. Arnold, ‘MIPoD: A Hypothesis-Testing Framework for Microevolutionary Inference from Patterns of Divergence’

(Am. Nat., vol. 171, no. 3, p. 366)

Construction of the Phylogenetic Divergence Matrix

To further illustrate the construction of the phylogenetic divergence matrix \mathbf{A} , we combine the \mathbf{G} matrix shown in figure 1A and the phylogeny shown in figure 2A. We thus have $m = 2$ traits and $n = 3$ taxa, so \mathbf{A} will be a 6-by-6 matrix. First, the \mathbf{G} matrix shown in figure 1A is parameterized by

$$\begin{pmatrix} \Sigma \\ \varepsilon \\ \varphi \end{pmatrix} = \begin{pmatrix} 5 \\ 0.6 \\ 0.8 \end{pmatrix}, \quad (\text{A1})$$

so the 2-by-2 \mathbf{G} matrix is

$$\mathbf{G} = \begin{bmatrix} 2.49 & 0.50 \\ 0.50 & 2.51 \end{bmatrix}. \quad (\text{A2})$$

The matrix of shared ancestry \mathbf{T} for the taxa x , y , and z is

$$\mathbf{T} = \begin{bmatrix} t_{xx} & t_{xy} & t_{xz} \\ t_{yx} & t_{yy} & t_{yz} \\ t_{zx} & t_{zy} & t_{zz} \end{bmatrix} = \begin{bmatrix} 1,000 & 200 & 200 \\ 200 & 1,000 & 800 \\ 200 & 800 & 1,000 \end{bmatrix}, \quad (\text{A3})$$

with time in generations. Each element in \mathbf{T} becomes a 2-by-2 block in \mathbf{A} by using equation (3), with $N_e = 100$, as follows:

$$\mathbf{A} = \begin{bmatrix} (1,000/100)\mathbf{G} & (200/100)\mathbf{G} & (200/100)\mathbf{G} \\ (200/100)\mathbf{G} & (1,000/100)\mathbf{G} & (800/100)\mathbf{G} \\ (200/100)\mathbf{G} & (800/100)\mathbf{G} & (1,000/100)\mathbf{G} \end{bmatrix}. \quad (\text{A4})$$

Thus, the final 6-by-6 \mathbf{A} matrix is

$$\mathbf{A} = \begin{bmatrix} 24.85 & 5.00 & 4.97 & 1.00 & 4.97 & 1.00 \\ 5.00 & 25.15 & 1.00 & 5.03 & 1.00 & 5.03 \\ 4.97 & 1.00 & 24.85 & 5.00 & 19.88 & 4.00 \\ 1.00 & 5.03 & 5.00 & 25.15 & 4.00 & 20.12 \\ 4.97 & 1.00 & 19.88 & 4.00 & 24.85 & 5.00 \\ 1.00 & 5.03 & 4.00 & 20.12 & 5.00 & 25.15 \end{bmatrix}. \quad (\text{A5})$$

For example, the variance of the distribution of the expected mean of trait 1 in taxon x is 24.85, the covariance between trait 1 in taxon x and trait 1 in taxon y is 4.97, and the covariance between trait 1 in taxon x and trait 2 in taxon z is 1.00.

The vector of trait means at the root of the phylogeny in figure 2A is

$$\bar{\mathbf{z}}_0 = (0, 0). \quad (\text{A6})$$

Therefore, under a neutral model of evolution by drift, the vector of expected trait means across all taxa at the tips of the phylogeny is given by

$$\boldsymbol{\mu}' = (0, 0, 0, 0, 0, 0). \quad (\text{A7})$$

The population trait means at the tips of the phylogeny are thus a sample from the six-dimensional multivariate normal distribution with mean vector $\boldsymbol{\mu}$ and variance/covariance matrix \mathbf{A} (eq. [10]).

Table A1

Estimates of species and regional population means for body and tail vertebral counts in *Thamnophis* and *Nerodia*

Species	Subspecies or population	Body	Tail	<i>n</i>	Reference
<i>T. melanogaster</i>	<i>chihuahuensis</i>	145.20	72.50	10	Tanner 1959
<i>T. melanogaster</i>	<i>canescens</i>	143.70	60.50	37	Conant 1963
<i>T. validus</i>	<i>celaeno</i>	141.10	71.00	64	Conant 1969
<i>T. validus</i>	<i>thamnophisoides</i>	133.60	68.70	63	Conant 1969
<i>T. errans</i>	Chihuahua and Durango	158.73	73.30	11	Smith 1942; Tanner 1959
<i>T. exsul</i>	Coahuila and Nuevo Leon	145.00	53.40	8	Rossman et al. 1989
<i>T. godmani</i>	Rangewide	141.75	65.56	12	Smith 1942
<i>T. mendax</i>	Rangewide	141.50	58.00	7 ^a	Rossman et al. 1996
<i>T. sumichrasti</i>	Rangewide	152.50	62.50	...	Rossman et al. 1996
<i>T. scalaris</i>	Rangewide	140.00	59.79	15	Smith 1942
<i>T. nigronuchalis</i>	Rangewide	156.53	66.57	15	Tanner 1985
<i>T. rufipunctatus</i>	Rangewide	164.68	70.21	25	Tanner 1985
<i>T. atratus</i>	San Francisco Peninsula	147.00	71.00	57 ^a	Fox 1951
<i>T. elegans</i>	Lassen Co., CA	167.52	80.59	708	Arnold and Phillips 1999
<i>T. elegans</i>	Humboldt Co., CA	151.73	71.25	481	Arnold and Phillips 1999
<i>T. couchii</i>	Feather River, CA	174.00	82.00	16	Fitch 1940
<i>T. gigas</i>	San Joaquin Valley, CA	161.00	73.00	13	Fitch 1940
<i>T. ordinoides</i>	Del Norte Co., CA	144.93	63.63	128	Fox 1948
<i>T. hammondii</i>	San Diego Co., CA	162.60	73.00	62 ^a	Fitch 1940; McGuire and Grismer 1993
<i>T. brachystoma</i>	Pennsylvania	136.60	59.30	34	Barton 1956
<i>T. butleri</i>	Ohio	137.50	56.60	19 ^a	Conant 1938
<i>T. radix</i>	<i>Radix</i>	151.00	65.00	400	Smith 1949
<i>T. radix</i>	<i>haydeni</i>	157.20	67.00	233	Smith 1949
<i>T. eques</i>	<i>virgatenuis</i>	157.26	71.97	57	Conant 1963
<i>T. eques</i>	<i>megalops</i>	157.50	71.13	48	Conant 1963
<i>T. marcianus</i>	<i>nigrolateris</i>	156.80	68.20	60	Mittelman 1949
<i>T. marcianus</i>	<i>marcianus</i>	149.10	68.60	79	Mittelman 1949
<i>T. cyrtopsis</i>	<i>collaris</i>	153.40	88.70	61 ^a	Webb 1966
<i>T. pulchrilatus</i>	Rangewide	162.70	76.10	15 ^a	Webb 1966
<i>T. cyrtopsis</i>	<i>cyrtopsis</i>	167.90	78.30	196 ^a	Milstead 1953
<i>T. chryseocephalus</i>	Rangewide	145.00	73.00	...	Rossman et al. 1996
<i>T. fulvus</i>	Rangewide	140.69	62.73	40	UMMZ, UTA
<i>T. proximus</i>	Rangewide	163.89	100.86	782	Rossman 1963
<i>T. sauritus</i>	Rangewide	156.75	112.08	549	Rossman 1963
<i>T. sirtalis</i>	Lassen Co., CA	160.12	78.73	199	OSCMNH
<i>T. sirtalis</i>	Humboldt Co., CA	151.70	79.95	268	OSCMNH
<i>N. erythrogaster</i>	<i>neglecta</i>	152.60	65.00	7	Conant 1949

Table A1 (Continued)

Species	Subspecies or population	Body	Tail	<i>n</i>	Reference
<i>N. erythrogaster</i>	<i>flavigaster</i>	150.00	66.60	8	Conant 1949
<i>N. fasciata</i>	North Carolina and Virginia	129.57	69.50	213	Osgood 1978

Note: Sample sizes (*n*) are numbers of female specimens. Midpoints of ranges, rather than means, are reported for *T. chrysocephalus*, *T. mendax*, and *T. sumichrasti*. Data for *T. fulvus* and *T. sirtalis* were taken from museum specimens (UMMZ, University of Michigan; UTA, University of Texas, Arlington; OSCMNH, Oregon State University).

^a Cases in which only the total number of both sexes was reported, so the number of female specimens was estimated by dividing the total sample size in half.

Table A2

Results of hypothesis testing with the garter snake test case, starting from four different direct estimates of **G** and a single estimate of **P**

Additional ML parameter estimate	G matrix parameters			lnL(θ)	LR	df	P value
	Σ	ϵ	φ				
<i>Thamnophis elegans</i> mean G matrix:							
None	15.51	.629	.843	-408.37
Size (Σ)	.308 (.228-.428)	.629	.843	-293.71	229.33	1	<.0001
Shape (ϵ)	.338 (.251-.471)	.793 (.677-.862)	.843	-290.57	6.28	1	.0122
Orientation (φ)	.338 (.251-.471)	.871 (.793-.914)	.513	-283.20	14.74	1	.0001
<i>T. elegans</i> coastal G matrix:							
None	14.68	.534	1.376	-407.62
Size (Σ)	.342 (.254-.477)	.534	1.376	-299.14	216.96	1	<.0001
Shape (ϵ)	.338 (.251-.471)	.442 (.306-.595)	1.376	-298.47	1.33	1	.248
Orientation (φ)	.338 (.251-.471)	.871 (.793-.914)	.513	-283.20	30.55	1	<.0001
<i>T. elegans</i> inland G matrix:							
None	16.33	.731	.785	-406.99
Size (Σ)	.304 (.226-.423)	.731	.785	-289.90	234.17	1	<.0001
Shape (ϵ)	.338 (.251-.471)	.817 (.712-.878)	.785	-288.73	2.35	1	.125
Orientation (φ)	.338 (.251-.471)	.871 (.793-.914)	.513	-283.20	11.06	1	.0009
<i>Thamnophis sirtalis</i> G matrix:							
None	7.44	.851	.993	-369.53
Size (Σ)	.468 (.347-.652)	.851	.993	-298.19	142.67	1	<.0001
Shape (ϵ)	.338 (.251-.471)	.712 (.569-.807)	.993	-294.87	6.64	1	.0100
Orientation (φ)	.338 (.251-.471)	.871 (.793-.914)	.513	-283.20	23.35	1	<.0001
<i>T. elegans</i> mean P matrix:							
None	30.59	.636	.770	-434.33
Size (Σ)	.301 (.224-.419)	.636	.770	-292.73	283.19	1	<.0001
Shape (ϵ)	.338 (.251-.471)	.822 (.720-.882)	.770	-288.26	8.95	1	.0028
Orientation (φ)	.338 (.251-.471)	.871 (.793-.914)	.513	-283.20	10.12	1	.0015

Note: Direct estimates of the parameters of the **G** matrix or **P** matrix are shown in the first row of each analysis (all estimates for *T. elegans* from Arnold and Phillips 1999; estimate for *T. sirtalis* from Dohm and Garland 1993). In each subsequent row, parameter values in bold were estimated by maximum likelihood (ML) from divergence data. The remaining parameters were fixed at the direct estimates. Marginal 95% confidence intervals around ML estimates for Σ and ϵ are given in parentheses. Estimates of Σ are scaled to $\bar{N}_e = 500$; lnL(θ) gives the log_e likelihood of the parameter values in each row of the table. LR gives the likelihood ratio test statistic (LR = 2 × [lnL(θ_i) - lnL(θ_o)]), where subscripts denote rows in the table. The statistical significance of LR (P value) was evaluated with a χ^2 distribution with the indicated degrees of freedom (df).

Table A3

Results of hypothesis testing of the effect of two taxa and the alignment of \mathbf{d}_{\max}^* with estimates of ω_{\max} from two different selection surfaces

Alternative hypothesis	G matrix parameters			$\ln L(\theta)$	LR	df	P value
	Σ	ε	φ				
ML best fit	.338 (.251–.471)	.871 (.793–.914)	.513	–283.20
Reduced data set best fit	.331 (.244–.465)	.910 (.854–.941)	.473	–285.11	3.83	3	.2804
<i>Thamnophis elegans</i> ω_{\max}	.338 (.251–.471)	.866 (.786–.911)	.430	–283.82	1.25	1	.2638
<i>Thamnophis radix</i> ω_{\max}	.338 (.251–.471)	.804 (.693–.869)	.817	–289.76	13.12	1	.0003

Note: The ML best fit represents the maximum likelihood estimate of all three parameters (with marginal 95% confidence limits for Σ and ε) using the full garter snake data set. The remaining rows of the table each represent a likelihood ratio test against the parameter values in the first row. The reduced data set best fit represents the ML estimate of all three parameters to the data set with *Thamnophis proximus* and *Thamnophis sauritus* removed. In the last two rows, φ was held constant at the direct estimate from ω_{\max} estimated from growth rate in *T. elegans* and crawling speed in *T. radix*. All parameter values in bold were estimated by maximum likelihood, while others were held constant. Statistics as in table A2.

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