

Processes driving male breeding colour and ecomorphological diversification in rainbow skinks: a phylogenetic comparative test

Gaynor Dolman · Devi Stuart-Fox

Received: 4 August 2008 / Accepted: 15 January 2009
© Springer Science+Business Media B.V. 2009

Abstract We used a phylogenetic comparative approach to investigate the importance of ecological shifts in the diversification of both signalling traits and ecomorphological traits in a diverse group of Australian skinks (*Carlia*). First, we tested whether divergence in male breeding coloration is associated with shifts in habitat openness. Second, we examined whether the type or location of male breeding coloration changes predictably with habitat openness. Third, we tested the ecomorphological predictions that body size should vary in relation to habitat openness and that limb length, toe length and head depth should vary with substrate use. Divergence in male breeding coloration was positively associated with shifts in habitat openness. Our results also indicate that species occupying more open habitats tend to use male sexual signals located on lateral body regions and not necessarily on body regions that are potentially more concealed from aerial predators (e.g. chest and throat). With regard to ecomorphological traits, habitat openness appears to have no predictable influence on body size at the inter-specific level, contrary to expectations based on intra-specific studies. However, consistent with functional predictions, we found that preference for rocky habitats is associated with relatively longer hind limb length, presumably due to selection for greater speed and jumping ability on these substrates. Overall, results of this study support the hypothesis that ecological shifts play a central role in promoting morphological diversification.

G. Dolman
School of Integrative Biology, University of Queensland, St Lucia, QLD 4072, Australia

G. Dolman
Australian National Wildlife Collection, CSIRO Sustainable Ecosystems, Canberra, ACT 2601, Australia

D. Stuart-Fox
Department of Zoology, University of Melbourne, Melbourne 3010, Australia

G. Dolman (✉)
Australian National Wildlife Collection, c/o School of Earth and Environmental Sciences, University of Adelaide, Darling Building, DX 650 418, North Terrace Campus, Adelaide, SA 5005, Australia
e-mail: gaynor.dolman@csiro.au

Keywords *Carlia* · Ecological speciation · Sexual signals · Ecomorphology · Comparative test

Introduction

Identifying the factors driving morphological diversification is a central goal of evolutionary biology. One of the most important of these factors is the colonisation of new environments or exploitation of new ecological niches (Muller 1942; Mayr 1947; Endler 1977; Schluter 2000). There is now a large body of evidence showing that ecological shifts have predictable consequences for morphological evolution (Wainwright and Reilly 1994; Schluter 2000). Ecological shifts (either in space or time) can lead to divergence in functional ecomorphological traits such as body size and shape as well as signalling traits, such as conspicuous colour patterns and ornaments. Few studies, however, have assessed the role of natural selection in the diversification of both signalling and ecomorphological traits.

In terms of signalling traits, habitat characteristics will influence the type and abundance of visual predators as well as the relative vulnerability of animals to predators due to differences in conspicuousness or shelter availability (Endler 1992, 1993). This, in turn, will affect the relative cost of sexual signals, potentially leading to divergence in these traits between habitats (Endler and Basolo 1998). Natural selection via predation pressure can influence not only the type of colour patterns or ornaments but their location as well. For example, in habitat with higher predation, conspicuous signals may be located on 'concealed' body regions such as the throat rather than on body regions more easily visible to predators (e.g. Stuart-Fox and Ord 2004; Ord and Stuart-Fox 2006). A second way in which habitat characteristics can influence the evolution of signalling traits is via their effect on signal detectability. Both the structural complexity of habitats and the associated light environment will affect the detectability of signals to conspecifics, potentially influencing both male–male interactions and mate choice decisions (Marchetti 1993; Boughman 2001; Fuller et al. 2005). This idea has been formalised in the 'sensory drive hypothesis', which proposes that the interaction between habitat-mediated signal detectability and sexual selection can promote divergence in signalling traits (Endler and Basolo 1998; Boughman 2002). This hypothesis predicts that the environment will have predictable consequences for the evolution of sexual signals because male sexual signals and female perception coevolve to optimise communication in a given environment (Endler 1992).

For ecomorphological traits such as body size and shape, habitat characteristics may impose strong selection either through abiotic features such as light regime, solar radiation, rainfall and productivity, or through its relationship to biotic interactions, such as predation pressure (Brown and Thorpe 1991; Garland and Losos 1994; Malhotra and Thorpe 1997; Schneider et al. 1999). For example, for most vertebrates predation pressure is assumed to be greater in more open habitats and may influence body size through selection on life history variables (Charlesworth 1994; Reznick et al. 1996; Schneider et al. 1999). Similarly, substrate type may also exert selection pressures on morphological dimensions through its influence on locomotor behaviour and microhabitat use. In ground-dwelling lizards, for instance, species occupying rocky habitats are predicted to have greater relative limb and toe lengths (Pianka 1969; Jackson 1973; Jaksic and Núñez 1979; Brown and Thorpe 1991; Vitt et al. 1997) associated with greater running and jumping ability (Garland

and Losos 1994) and reduced relative head depth associated with use of rock crevices for shelter (Vitt et al. 1997; Arnold 1998; Revell et al. 2007; Goodman and Isaac 2008).

Here, we use a phylogenetic comparative approach to test hypotheses explaining diversification of both signalling traits (male breeding colour) and ecomorphological traits (body size and shape) in the *Carlia* group of lizards. *Carlia* (*s.l.*) is the most diverse clade of *Eugongylus*-group skinks in Australia (Greer 1989; Cogger 2000). *Carlia* (*s.l.*) are collectively known as ‘rainbow skinks’ due to the presence of bright colours on breeding males of many species. Based on recent phylogenetic work (Dolman and Huggall 2008), the group comprises three closely related genera: *Carlia* (Gray, 1845); *Lygisaurus* (De Vis, 1884); and *Liburnascincus* (Wells and Wellington, 1984) which together we refer to as the ‘*Carlia* group’. The *Carlia* group comprises small to moderately large ground-dwelling or rock-climbing skinks (Ingram and Covacevich 1989) and is distributed across tropical and subtropical eastern and northern Australia, New Guinea and surrounding islands. Though morphologically conservative in scale pattern and other taxonomically informative characters (Ingram and Covacevich 1988, 1989), the species are variable in size, body shape and type and location of bright colours in breeding males. Moreover, the species are found in diverse vegetation types, from tropical rainforest to semi-arid grasslands and on diverse substrates, from the sandy soils in coastal and desert areas to bare granite (Ingram and Covacevich 1988, 1989; Cogger 1996). This morphological and ecological diversity make *Carlia* an ideal group in which to test for processes driving their diversification. Furthermore, it has been suggested that ecological shifts are necessary for morphological diversification in *Carlia* (Schneider et al. 1999), which otherwise show long-term morphological stasis. This suggestion was based on evidence that across ecotones, *C. rubrigularis* and *C. rhomboidalis* were consistently smaller in more open than closed forest habitats, indicating that habitat differences exert strong selection on body size (Schneider et al. 1999; Smith et al. 2001). By contrast, there is no apparent morphological differentiation between two highly genetically divergent lineages of *C. rubrigularis* which occupy similar habitats (Schneider et al. 1999; Smith et al. 2001). An investigation of the processes promoting morphological diversification in the whole *Carlia* group may provide some broader insight into this hypothesis.

In this study, we examined the relationship between ecological shifts and divergence in male breeding coloration and other morphological traits, specifically body size, limb and toe lengths and head depth because these traits are most likely to vary in relation to habitat openness and substrate preference. We tested the prediction that shifts in habitat openness have led to predictable changes in the type or location of male breeding coloration. To examine the influence of ecological shifts on ecomorphological traits, we tested the predictions that body size should vary with habitat openness and that species preferring rocky substrates should have relatively longer hind legs and toes and relatively flatter head shape.

Methods

Data

We collected data for 34 species, which include all 31 currently described Australian species of the *Carlia* group and representatives from each of the *Carlia fusca*-, *Carlia bicarinata*-, and *Lygisaurus novaeguineae*-complexes, which are distributed in New Guinea and surrounding islands. For taxonomic detail of specimens used in this study, including explanations for continued taxonomic ambiguity of specimens from *C. fusca*-

and *L. novaeguineae*-complexes following recent revisions of these groups (Zug 2004; Kraus 2007), refer to Dolman and Hugall (2008). We noted from the literature (Storr 1974; Ingram and Covacevich 1988, 1989; Whittier 1993) whether each species was present or absent for three broad vegetation classes and three substrate classes as follows: vegetation: open forest/savannah, monsoon/scrub (intermediate canopy cover) or closed forest; substrate: boulder or stony substrate, sandy substrate or clay/loam substrate. These categories were chosen because they represent the habitat features which are likely to directly influence lizard morphology and could be unambiguously scored from the literature (Cogger 2000, Ingram and Covacevich 1988, 1989). From these classifications we derived scores for ‘habitat openness’ and substrate preference (Table 1). We scored habitat openness by deriving a ranked score from 0 to 4: 0 = closed habitats only; 1 = closed + intermediate habitats; 2 = intermediate habitats only; 3 = intermediate + open and 4 = open habitats only. In terms of substrate preference, the great majority of *Carlia* species occur in leaf-litter on clay/loam or sandy substrates or are generalists, occurring in leaf-litter on most substrates including rocky and stony ranges. However, several species are rocky habitat specialists and/or active climbers. Therefore we classified substrate preference as a binary variable (1 = preference for rocky habitat/active climber; 0 = other substrates). As *C. fusca* sp. and *L. novaeguineae* sp. specimens used in this study remain taxonomically ambiguous following these reviews of Zug (2004) and Kraus (2007) (see Dolman and Hugall 2008 for details), habitat openness was scored based on collection location details of the individual specimens.

In *Carlia* breeding male coloration is found on a number of regions of the body. We collated information on coloration of breeding males from species descriptions in Cogger (2000) and the detailed taxonomic revisions of Ingram and Covacevich (1988, 1989) (Table 1). We allocated these body regions into four categories: (1) throat/neck/chest; (2) lateral region/flanks; (3) head and (4) limbs or tail. For each body region we noted whether male coloration was primarily long wavelength (reds and oranges), medium wavelength (green), short wavelength (blues) or achromatic (black or white). Variation in male breeding colour reported in recent revisions of the *C. fusca*-complex (Zug 2004) fell within character state categories assigned to *C. fusca* sp. Similarly, although ethanol preservation prohibited descriptions of male breeding colour in most species of the *L. novaeguineae* complex (Kraus 2007), variation between previously confounded taxa is likely to be within character state categories assigned to these taxa. Some *Carlia* species are known to show reflectance in the ultraviolet (Blomberg et al. 2001), which is not visible to humans. However, almost all natural colours with a UV component also show reflectance in the human-visible part of the spectrum. For this reason, it is unlikely that divergence in male breeding coloration occurs purely in the ultraviolet, suggesting that any colour divergence is likely to be apparent to humans.

We measured morphological traits from museum specimens for a total of 1,259 individuals representing 29 of the 34 species. Each individual was dissected to determine its sex and to ascertain whether it was sexually mature. Morphological measurements were only taken for mature individuals. For the majority of species, between 20 and 35 individuals of each sex were measured. Exceptions due to limited numbers of museum specimens were *C. bicarinata* (30 total) *C. dogare* (29 individuals total), *C. coensis* (11 total), *C. fusca* (20 total), *L. macfarlani* (30 total), *L. parrhasius* (6 total), *L. sesbrauna* (22 total), *L. tanneri* (6 total) and *L. zuma* (15 total). We measured the following morphological variables to 0.01 mm with callipers: snout-vent length (SVL), hind leg length (from the tip of the longest toe to the point of insertion of the limb to the body wall); hind toe length (length of the longest toe–fourth hind toe) and head depth (depth of the head taken

Table 1 Data used in phylogenetic comparative analyses

Species	Openness (ranked)	Rocky substrate	Colour—throat/neck	Colour—lateral	Colour—limbs/tail	Colour—short wavelength	SVL	Relative head depth	Relative hind limb length	Relative hind toe length
<i>C. rhomboidalis</i>	1	0	1	0	0	1	49.58	-0.22	-0.953	-0.314
<i>C. rubrigularis S</i>	0	0	1	0	0	0	48.79	-0.183	-0.443	-0.214
<i>C. rubrigularis N</i>	0	0	1	0	0	0	48.79	-0.183	-0.443	-0.214
<i>C. dogare</i>	3	0	0	1	0	0	43.74	0.233	4.313	1.79
<i>C. vivax</i>	4	0	0	1	0	0	39.255	-0.143	1.205	1.025
<i>C. rostralis</i>	3	0	1	1	0	1	56.295	-0.369	-0.758	0.456
<i>C. pectoralis</i>	3	0	0	1	0	0	42.275	0.135	1.138	0.475
<i>C. munda</i>	3	0	1	1	0	0	37.735	-0.08	0.805	0.304
<i>C. johnstonei</i>	2	0	0	0	1	1	38.09	0.206	0.776	-0.209
<i>C. triacantha</i>	4	0	0	1	0	1	42.94	0.117	1.729	0.592
<i>C. amax</i>	3	1	0	1	0	1	36.685	0.2	1.437	0.38
<i>C. rufilatus</i>	3	0	0	1	0	1	38.27	-0.079	0.975	0.53
<i>C. tetradactyla</i>	4	0	1	1	0	1	54.435	0.021	-5.238	-1.675
<i>C. longipes</i>	3	0	0	1	0	1	53.86	0.174	0.517	1.042
<i>C. fusca</i> sp.	3	0	0	1	0	0	59.458	1.009	-4.378	-0.136
<i>C. bicarinata</i>	4	0	0	1	0	0	42.125	0.565	-0.75	0.495
<i>C. storri</i>	3	0	0	0	1	0	40.195	0.044	1.594	0.703
<i>C. schmeltzi</i>	3	0	0	1	0	0	58.72	-0.121	-3.397	-0.429
<i>C. jarnoldae</i>	4	1	2	1	1	0	40.31	0.286	1.51	0.423
<i>C. gracilis</i>	1	0	0	1	1	0	36.255	-0.112	0.555	0.502
<i>Ly. abscondita</i>	4	0	1	0	1	0				
<i>Ly. foliorum</i>	3	0	1	0	1	0	29.87	-0.134	-2.373	-0.737
<i>Ly. tanneri</i>	3	0	1	0	1	0	31.68	0.056	-2.39	-1.298
<i>Ly. malleolus</i>	4	0	1	0	1	0				
<i>Ly. zuma</i>	3	0	1	0	1	0	30.859	-0.3	0.377	-0.46

Table 1 continued

Species	Openness (ranked)	Rocky substrate	Colour—throat/neck	Colour—lateral	Colour—limbs/tail	Colour—short wavelength	SVL	Relative head depth	Relative hind limb length	Relative hind toe length
<i>Ly. parthasius</i>	4	1	0	0	1	0	30.49	-0.173	2.067	0.563
<i>Ly. laevis</i>	2	0	1	0	1	0				
<i>Ly. aertatus</i>	2	0	1	0	1	0				
<i>Ly. novaequinae</i> sp.	0	0	0	0	1	0				
<i>Ly. novaequinae</i> sp.	2	0	1	0	1	0	31.725	-0.029	-3.214	-1.106
<i>Ly. sesbrauna</i>	3	0	0	0	1	0	30.371	-0.024	-2.447	-0.922
<i>Li. coensis</i>	3	1	0	0	0	0	57.785	-0.398	2.951	-0.113
<i>Li. scirtetis</i>	4	1	0	0	0	0	61.19	-0.444	5.848	-0.306
<i>Li. mundivensis</i>	4	1	0	0	0	0	51	-0.056	-1.013	-1.146

For binary variables, zero and one denote absence or presence of the trait or preference for the substrate type. Habitat openness is ranked from 0 to 4 with 0 representing closed habitats and 4 representing open habitats—see text for additional detail

at the parietal scales). Morphological characters were corrected for body size by calculating the residuals of each variable regressed against snout-vent length (SVL). We used species mean values in all comparative analyses (Table 1).

Phylogenetic comparative analyses

We used the phylogeny of Dolman and Hugall (2008; Fig. 1), based on mitochondrial loci (726 bp *ND4*, 473 bp 16S) and two nuclear intron loci (342 bp *Aldolase*, 806 bp *β -globin*, and 12 and 133 presence/absence characters of *Aldolase* and *β -globin* indels, respectively). Phylogenetic reconstruction was based on partitioned Bayesian analysis performed in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). As a consequence of the group having undergone a period of rapid diversification at an intermediate stage during its evolution, the phylogeny contains 9 nodes with less than 90% Bayesian Posterior Support (Fig. 1). We accounted for phylogenetic uncertainty in two ways, depending on the analysis. For phylogenetic least squares regression, we repeated analyses on 100 trees drawn at random from the MrBayes search and derived 95% confidence intervals around regression slopes based on these 100 trees. For sister clade comparisons, we only used sister clades with >90% Bayesian posterior support. Of these, all but one node had posterior support >95%. As the exception was a node with a posterior support of 94% which was not subject to gene conflict, we included it to maximise statistical power. However, we repeated analyses with and without the node and results remained qualitatively unchanged. All trees were converted into ultrametric trees using Penalized Likelihood Rate Smoothing (PRLS) in the program r8s v1.7 (Sanderson 2002, 2004). To ensure that taxa in the tree exactly matched those for which we had comparative data, trees were pruned to include only ingroup taxa and one representative from each species (except for *C. rubrigularis* which is paraphyletic with *C. rhomboidalis*, and is therefore represented by each of two major lineages). For analyses involving morphometric measurements, we pruned the tree to include only the 29 taxa for which we had morphological data. Molecular phylogenetic analyses of restricted sampling of *C. fusca*-, *C. bicarinata*- and *L. novaeguineae*-complexes suggest that they form three separate monophyletic lineages and therefore unsampled taxa within these complexes represent missing data at the tips of the phylogeny and are not likely to affect nodes used in these analyses. Likewise morphometric measurements encompass variation present at the tips of each of these lineages.

First, we tested whether divergence of male breeding colour pattern may have been driven by shifts in habitat openness. We tested for pairwise correlations between degree of divergence in male breeding colour pattern and divergence in habitat openness. Presence and absence of male breeding colour for each wavelength category and each body region, and habitat openness ranked scores were mapped onto extant taxa. Ancestral states were reconstructed using maximum likelihood in Mesquite version 2.5 (Maddison and Maddison 2008). The Asymmetrical Markov k-state 2 parameter model (suitable for data with two character states) was used for the colour data and the Markov k-state 1 parameter model (suitable for multi-state data) was used for the habitat data. The level of divergence in male breeding colour between each sister pair was summarised as the number of changes in body position or wavelength. Where ancestral reconstruction of colour was uncertain, we counted no change. In all but one case, summaries of colour divergence were based on three or four well-supported character states (one pair having only two). Shifts in habitat openness for each sister pair were summarised by taking the difference in openness rank scores between sister pairs. Where

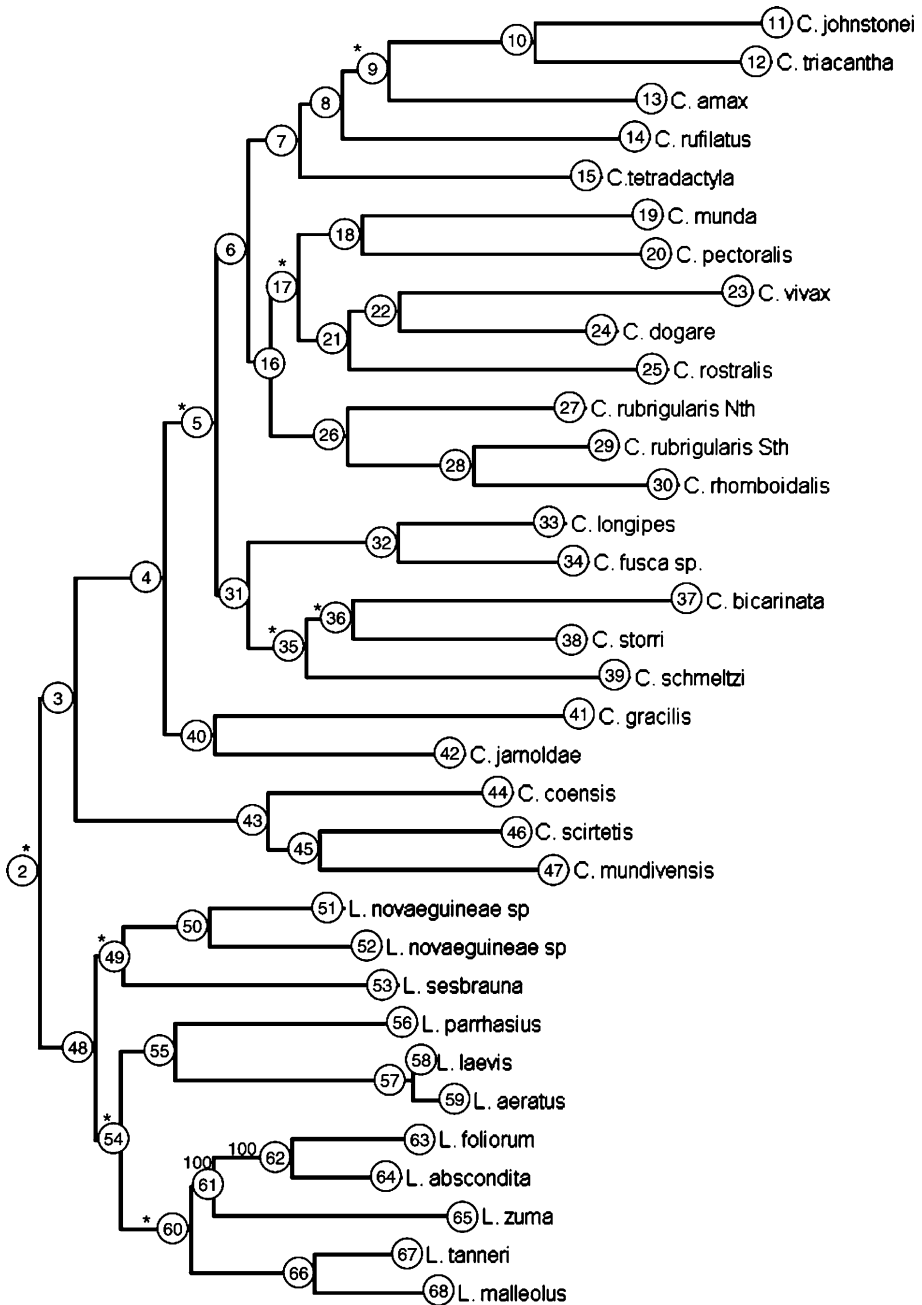


Fig. 1 Bayesian all compatible consensus phylogram with labelled nodes (refer to Table 1) and posterior probabilities <90% highlighted with “*”

ancestral reconstruction of habitat rank was uncertain, sister pairs were omitted from the correlation (28 pairs included, 5 omitted). Spearman's rank correlation tests were conducted on these differences in colour and habitat.

Next, we tested for evolutionary correlations between habitat and morphology. Specifically, we tested whether (1) shifts in habitat openness were accompanied by changes in the type or location of male breeding coloration; (2) there were correlated changes in habitat openness and body size and (3) whether shifts in substrate preferences were accompanied by changes in head dimensions and relative hind limb and toe lengths. For these analyses, we used Phylogenetic Generalised Least Squares (PGLS) regression (Martins and Hansen 1997). PGLS estimates a parameter (α) for each correlation or regression that can be interpreted as phylogenetic constraint on a phenotypic trait or a measure of phylogenetic inertia (Hansen 1997; Martins et al. 2002; Butler and King 2004; Hansen and Orzack 2005). When the parameter α is set to 0, PGLS produces results identical to Felsenstein's independent contrasts (FIC). When α is large, it is equivalent to ignoring phylogeny or assuming all species radiated simultaneously from a single common ancestor (TIPS) (Martins et al. 2002, 2004).

For the analysis of location of male breeding coloration we only included three body regions (throat, lateral and limbs or tail) and did not include the head as only three species showed coloration on the top of the head. In terms of the type of breeding coloration, only two species possess medium wavelength breeding coloration and all but three species possess long wavelength colours. Eight species possessed short wavelength breeding colours, providing sufficient variability for analysis. We therefore tested whether presence of short wavelength breeding colours was associated with habitat openness. To identify habitat characteristics that predicted type and location of male breeding colour and morphological traits, we constructed 95% confidence intervals around regression slopes (Martins 1994; Ord and Stuart-Fox 2006). Regression analyses were done using the program COMPARE v4.6 (Martins 2004).

Results

Members of the *Carlia* group showed numerous evolutionary changes in both male breeding coloration and habitat (Table 2). Based on sister pair comparisons, we tested for pairwise correlations between shifts in habitat openness and divergence in male breeding colour pattern. The level of difference in habitat openness ranked scores was positively correlated with the level of divergence in colour pattern ($r_s = 0.53$, $df = 26$, $P = 0.006$). Accounting for phylogenetic uncertainty by removing nodes of less than 90% Bayesian Posterior Support resulted in a similar trend ($r_s = 0.48$, $df = 14$, $P = 0.032$).

PGLS analyses revealed that ecological shifts result in predictable shifts in both the location of male breeding coloration and morphological dimensions. For each regression, we present the confidence limits around the slope for the best phylogeny in the text and frequency distributions of the upper and lower confidence limits for 100 possible trees in an accompanying figure (Fig. 2, 3). If the confidence limits do not incorporate 0 then the slope of the regression (β) can be considered significantly different from 0.

There was no relationship between habitat openness and presence of breeding coloration on the throat (95% CI = $-0.17 < \beta < 0.07$, % $r^2 = 0.61$; Fig. 2a). However, there was a positive relationship between habitat openness and occurrence of breeding coloration on the lateral region. Species occupying more open habitats tended to have breeding colours on the lateral region (95% CI = $0.03 < \beta < 0.23$, % $r^2 = 16.71$; Fig. 2b). Presence of coloration on

Table 2 Data used in sister pair comparisons

	Ancestral node	Sister pairs		Colour divergence	Habitat openness
		Node 1	Node 2	Total # changes	Rank score difference
	2	48	3	2	0
	3	43	4	2	?
	4	40	5	0	?
	5	31	6	0	0
	6	16	7	0	0
	7	15	8	0	2
	8	14	9	0	2
	9	13	10	2	?
	10	11	12	2	2
	16	26	17	2	?
	17	21	18	0	0
	18	19	20	0	0
	21	25	22	0	0
	22	23	24	2	1
	26	28	27	0	0
	28	29	30	2	1
	31	35	32	0	0
	32	34	33	2	0
	35	39	36	0	0
	36	38	37	3	1
	40	42	41	2	3
	43	45	44	0	1
	45	47	46	0	0
	48	54	49	0	0
	49	53	50	0	0
	50	52	51	3	2
See phylogeny in Fig. 1 for node labels	54	60	55	0	?
“?” ML ancestral state reconstruction of rank openness could not be assigned with confidence	55	57	56	0	2
	57	58	59	2	0
Nodes with >90% Bayesian Posterior Support are highlighted in bold	60	66	61	0	0
	61	65	62	0	0
	62	63	64	0	1
	66	67	68	0	1

the limbs or tail was not associated with habitat openness (95% CI = $-0.16 < \beta < 0.05$, % $r^2 = 2.99$; Fig. 2c). Use of short wavelength breeding colours was similarly unrelated to habitat openness (95% CI = $-0.06 < \beta < 0.14$, % $r^2 = 2.01$; Fig. 2d).

Shifts in habitat openness also appear to have had no predictable effects on body size (95% CI = $-1.81 < \beta < 3.21$, % $r^2 = 1.15$; Fig. 3a). Shifts to preference for rocky substrates were not associated with changes in head depth (95% CI = $-0.37 < \beta < 0.17$, % $r^2 = 1.81$; Fig. 3b) or hind toe length (95% CI = $-0.45 < \beta < 1.04$, % $r^2 = 2.28$; Fig. 3c) but were positively associated with a relative increase in hind limb length (95%

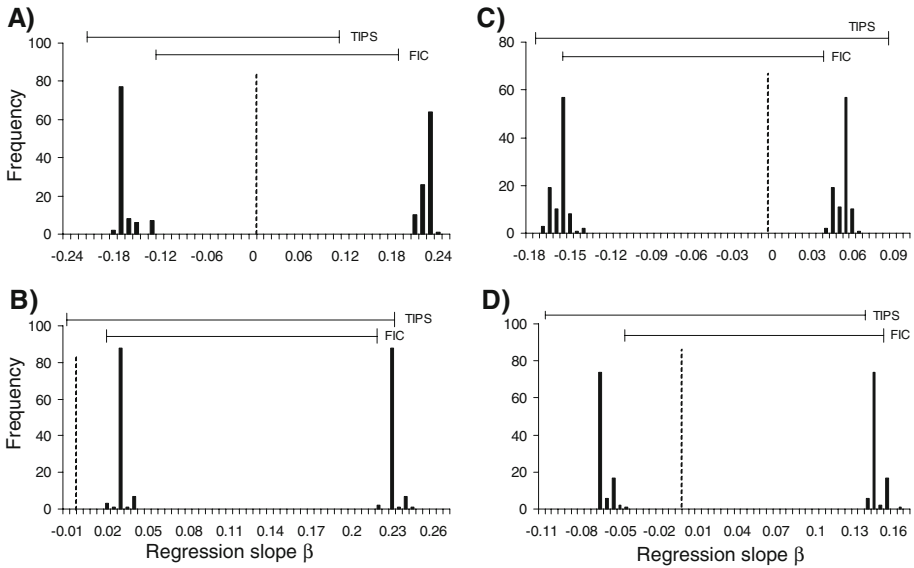


Fig. 2 The influence of habitat openness on location and type of male breeding coloration (a) presence of coloration on the throat; (b) coloration on the lateral region; (c) coloration on the limbs or tail; (d) presence of short wavelength breeding colours. Frequency distributions are of 95% upper and lower confidence intervals (CI) around the regression slope from Phylogenetic Least Squares Regression (PGLS) on 100 possible trees. If the upper and lower confidence limits do not fall on either side of 0 (dashed vertical line) then the slope can be considered significantly different from 0. CIs using Felsenstein’s independent contrasts (FIC) and non-phylogenetic analysis (TIPS) based on the ‘best’ tree are shown as horizontal lines above

CI = $0.67 < \beta < 4.71$, % $r^2 = 20.15$; Fig. 3d). Thus, species preferring rocky substrates have longer legs but do not have relatively shorter toes or flatter head shape.

Discussion

We used a comparative phylogenetic approach to investigate processes promoting morphological diversification in the *Carlia* group of skinks. First, we tested whether divergence in male breeding coloration was associated with shifts in habitat openness. We found there was a pairwise correlation between degree of divergence in male breeding colour pattern and divergence in habitat openness, providing evidence for a role for ecological shifts in driving diversification of male breeding coloration. Next, we tested whether shifts in habitat openness were accompanied by changes in the type or location of male breeding coloration. We found a positive relationship between habitat openness and occurrence of breeding coloration on the lateral region, with species occupying more open habitats tending to have breeding colours on lateral regions of their bodies. However, there was no correlation between habitat openness and presence of breeding coloration on the throat, limbs or tail or the use of short wavelength breeding colours. Finally, we tested for correlations between ecological shifts and divergence in ecomorphological traits. We found no evidence that shifts in habitat openness have predictable effects on body size. Shifts to preference for rocky substrates were, however, associated with a relative increase in hind limb length, although they had no predictable effects on head depth or hind toe length.

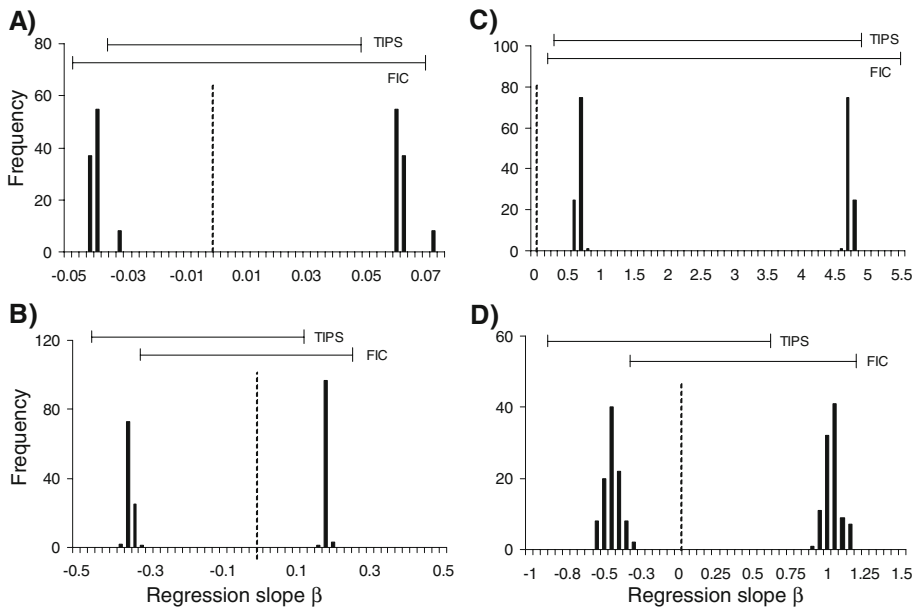


Fig. 3 The influence of (a) habitat openness on body size and the influence of preference for rocky substrate on (b) relative head depth; (c) relative hind limb length; (d) relative hind toe length. Frequency distributions are of 95% upper and lower confidence intervals (CI) around the regression slope from Phylogenetic Least Squares Regression (PGLS) on 100 possible trees. If the confidence limits do not fall on either side of 0 (dashed vertical line) then the slope can be considered significantly different from 0. CIs using Felsenstein's independent contrasts (FIC) and non-phylogenetic analysis (TIPS) based on the 'best' tree are shown as horizontal lines above

A significant correlation between shifts in habitat openness and divergence in male breeding colour suggests a role for ecological divergence in the diversification of *Carlia* skinks. Divergent predator-driven natural selection (Endler 1980; Langerhans et al. 2007) and sensory drive (Endler 1992) are two mechanisms that could explain this pattern. Natural selection via predation pressure can influence both the type and location of colour patterns. In habitats with higher predation, conspicuous signals may be located on 'concealed' body regions such as the throat rather than on body regions more easily visible to predators (e.g. Stuart-Fox and Ord 2004; Ord and Stuart-Fox 2006). Higher predation pressure from visual predators such as birds and diurnal, visually hunting snakes is predicted in more open habitat (e.g. Schneider et al. 1999; Gifford et al. 2008). Predators of *Carlia* include birds, for example, the laughing kookaburra (*Dacelo novaeguineae*) (Langkilde et al. 2003, 2005) but also include diurnal, visually hunting snakes likely to have well-developed colour vision (Sillman et al. 1997) such as whip snakes (*Demansia*) (Shine 1980). While the assumption of higher predation in more open habitats has received experimental support for one species, *C. rubrigularis* (Schneider et al. 1999), which occurs in rainforest and adjacent more open wet sclerophyll forests, it has not been tested over the broad spatial scale and range of habitats occupied by members of the *Carlia* group. The limited number of *Carlia* (*s.l.*) that have coloration on conspicuous body regions such as the head ($N = 2$), dorsal or dorso-lateral body regions ($N = 0$) prevented us from testing for an association with habitat openness and occurrence of breeding coloration on body regions clearly visible to aerial predators. However, we found that species occupying more

open habitats were more likely to have breeding colours on the lateral region of their bodies. Lateral body regions of litter-dwelling skinks are not necessarily exposed to aerial predators; therefore these results do not contradict predictions of predator-driven selection. However, the lack of association between habitat openness and male breeding coloration on the throat and chest suggests that, at this phylogenetic scale, there is little evidence that variation in predation pressure between habitats has influenced the location of male breeding coloration.

According to the sensory drive hypothesis, intra-specific signals, perceptual systems and behaviour should be evolutionarily coupled in order to maximise the signal to background noise ratio and to minimize signal degradation (Endler 1992, 1993; Boughman 2002). When populations experience different light environments in different habitats, colours that appear relatively conspicuous in one environment may appear much less conspicuous in another. In general, reds and oranges are most conspicuous in closed forest, while greens and blues appear most conspicuous in open forest (Endler 1992). At this phylogenetic scale, we found no evidence that use of short wavelength breeding colours (blues) was positively related to habitat openness, as predicted by the sensory drive hypothesis. Sensory drive may, however, have a role in the position of male breeding colours in the *Carlia* group. For example, coloration on lateral body regions may be more easily seen over longer signalling distances, which may covary with habitat openness. Further research on differences in signalling behaviour and detectability of species in closed and open habitats is required to explain why lateral male breeding coloration is more common in open habitats.

In terms of ecomorphological traits, our results show some evidence for adaptive morphological variation. We identified a positive relationship between relative hind limb length and preference for rocky habitats. This matches functional expectations as species with longer legs for their body size run faster and jump further (Garland and Losos 1994; Van Damme and Vanhooydonck 2001). These abilities are likely to be particularly important for predator avoidance in relatively exposed rocky habitats or for species that are active climbers. Contrary to our findings, a recent ecomorphological study of 18 tropical skink species, including 11 members of the *Carlia* group, found that rock dwelling species did not have relatively longer hind limbs but did have flatter heads (Goodman and Isaac 2008). This study, however, aimed to identify convergent morphology among species from five genera whereas our study focuses specifically on the *Carlia* group.

Our results suggest that at the generic level, ecological diversification in the *Carlia* group does not appear to have been accompanied by predictable evolution of body size, head depth or toe length. Variation in head and limb dimensions, particularly in relation to substrate use, is well documented (Pianka 1969; Jackson 1973; Jaksic and Núñez 1979; Greer 1989; Brown and Thorpe 1991; Vitt et al. 1997; Goodman and Isaac 2008) yet some studies have similarly failed to find positive associations between morphology and habitat (Vitt 1991; Miles 1994; Jaksic et al. 1980). One reason for failing to identify clear among species trends relating morphology and habitat use is that species within a single habitat category may exhibit different patterns of microhabitat exploitation. For example, Miles (1994) noted that the broad category of 'saxicolous substrate' used in his study of nine species of phrynosomatid lizard included crevice-dwelling species and species favouring talus slopes, but that the use of these habitats involved very different patterns of movement and hence required different morphological adaptations. In other words, there are alternative adaptive solutions to living in rocky habitats, so that a broad comparative approach does not detect consistent trends. A similar explanation can be advanced for the lack of a predicted correlation between body size and habitat openness or head depth or toe length

and substrate preference in the *Carlia* group because patterns of exploitation may vary considerably within the broad habitat categories used in this study. An alternative explanation is that variation in relation to coarse measures of habitat openness and substrate is not detectable at this phylogenetic level. Because morphological dimensions at the intra-generic level can be relatively conserved, differentiation along other niche axes such as activity time, prey type and microhabitat may be more important in the evolution of this group of skinks (e.g. Jaksic et al. 1980).

The lack of association between body size and habitat openness at this phylogenetic scale is also at odds with previous conclusions drawn from members of the *Carlia* group at a finer scale. *C. rubrigularis* and *C. rhomboidalis* were found to be consistently smaller in more open habitat than in adjacent closed habitat across ecotones (Schneider et al. 1999; Smith et al. 2001). Thus although important for generating intraspecific morphological variation, there is no evidence that this variation has commonly driven speciation in the evolutionary history of the *Carlia* group. Overall, however, our results support the view that ecological shifts are important in the diversification of both signalling and ecomorphological traits within this group. The finding that ecological shifts accompany morphological divergence (particularly male breeding coloration) is consistent with the suggestion that a lack of morphological differentiation between two highly genetically divergent lineages of *C. rubrigularis* subject to long-term refugial isolation may be due to niche conservatism within their historical rainforest refuges (Schneider et al. 1999).

This study has demonstrated that within the *Carlia* group, shifts in habitat openness are important in the divergence of sexual signalling traits and that occurrence in more open habitats is accompanied by use of male sexual signals located on lateral body regions. However, the potential role of predator-driven natural selection in explaining this pattern is equivocal and predictions regarding the direction of evolution of male breeding colour for optimal signalling and female perception (blues in more open habitat; Endler 1992) were not supported at this phylogenetic scale. Further investigation into differences in signalling behaviour and detectability of species in closed and open habitats is required. Unlike the case for signalling traits, shifts in habitat openness appear to have no predictable effect on body size. However, we found that preference for rocky habitats or active climbing is associated with relatively longer hind limb length, presumably due to selection for speed and jumping ability. These findings are compatible with the hypothesis that ecological shifts play an important role in promoting diversification of both signalling and ecomorphological traits. Additionally, this study provides a phylogenetic perspective on processes promoting morphological diversification, which has been a topic of interest at a finer scale in *Carlia* (Schneider et al. 1999; Smith et al. 2001; Dolman 2008).

Acknowledgments We thank Craig Moritz for guidance and partial funding. We are grateful to Adnan Moussalli for constructive comments and Craig Moritz, Sandie Degnan, Jeremy Austin and John Endler for helpful comments on drafts of an earlier manuscript. We are very grateful to Patrick Couper and Andrew Amey (Queensland Museum), Paul Horner (Northern Territory Museum), Ross Sadler (Australian Museum), Steve Donnellan (South Australian Museum) and Laurie Smith (West Australian Museum) for access to collections and insight into the biology of *Carlia*. GD was supported by an F. G. Meade Scholarship and a CSIRO OCE Postdoctoral Fellowship and DSF was supported by the Australian Research Council.

References

- Arnold EN (1998) Cranial kinesis in lizards—variations, uses, and origins. *Evol Biol* 30:323–357
- Blomberg S, Owens IPF, Stuart-Fox D (2001) UV reflectance in the small skink, *Carlia pectoralis*. *Herpetol Rev* 32:16–17

- Boughman JW (2001) Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* 411:944–948. doi:[10.1038/35082064](https://doi.org/10.1038/35082064)
- Boughman JW (2002) How sensory drive can promote speciation. *Trends Ecol Evol* 17:571–577. doi:[10.1016/S0169-5347\(02\)02595-8](https://doi.org/10.1016/S0169-5347(02)02595-8)
- Brown RP, Thorpe RS (1991) Within-Island microgeographic variation in body dimensions and scalation of the skink *Chalcides sexlineatus*, with testing of causal hypotheses. *Biol J Linn Soc Lond* 44:47–64. doi:[10.1111/j.1095-8312.1991.tb00606.x](https://doi.org/10.1111/j.1095-8312.1991.tb00606.x)
- Butler MA, King AA (2004) Phylogenetic comparative analysis: a modeling approach for adaptive evolution. *Am Nat* 164:683–695. doi:[10.1086/426002](https://doi.org/10.1086/426002)
- Charlesworth B (1994) The genetics of adaptation—lessons from mimicry. *Am Nat* 144:839–847. doi:[10.1086/285710](https://doi.org/10.1086/285710)
- Cogger HG (1996) Reptiles and amphibians of Australia. Reed Books Australia, Port Melbourne
- Cogger HG (2000) Reptiles and amphibians of Australia. Reed New Holland, Sydney
- Dolman G (2008) Evidence for differential assortative female preference in association with refugial isolation of rainbow skinks in Australia's tropical rainforests. *PLoSOne* 3:e3499. doi:[10.1371/journal.pone.0003499](https://doi.org/10.1371/journal.pone.0003499)
- Dolman G, Hugall AF (2008) Combined mitochondrial and nuclear data enhance resolution of a rapid radiation or Australian rainbow skinks (Scincidae: *Carlia*). *Mol Phylogenet Evol* 49:782–794. doi:[10.1016/j.ympev.2008.09.021](https://doi.org/10.1016/j.ympev.2008.09.021)
- Endler JA (1977) Geographic variation, speciation, and clines. Princeton University Press, Princeton
- Endler JA (1980) Natural selection on colour patterns in *Poecilia reticulata*. *Evol Int J Org Evol* 34:76–91. doi:[10.2307/2408316](https://doi.org/10.2307/2408316)
- Endler JA (1992) Signals, signal conditions, and the direction of evolution. *Am Nat* 139:S125–S153. doi:[10.1086/285308](https://doi.org/10.1086/285308)
- Endler JA (1993) Some general comments on the evolution and design of animal communication systems. *Philos Trans R Soc Lond B Biol Sci* 340:215–225. doi:[10.1098/rstb.1993.0060](https://doi.org/10.1098/rstb.1993.0060)
- Endler JA, Basolo AL (1998) Sensory ecology, receiver biases and sexual selection. *Trends Ecol Evol* 13:415–420. doi:[10.1016/S0169-5347\(98\)01471-2](https://doi.org/10.1016/S0169-5347(98)01471-2)
- Fuller RC, Houle D, Travis J (2005) Sensory bias as an explanation for the evolution of mate preferences. *Am Nat* 166:437–446. doi:[10.1086/444443](https://doi.org/10.1086/444443)
- Garland TJ, Losos JB (1994) Ecological morphology of locomotor performance in Squamate reptiles. In: Wainwright PC, Reilly SM (eds) Ecological morphology: integrative organismal biology. University of Chicago Press, Chicago, pp 240–302
- Gifford ME, Herrel A, Mahler DL (2008) The evolution of locomotor morphology, performance, and anti-predator behaviour among populations of *Leiocephalus* lizards from the Dominican Republic. *Biol J Linn Soc Lond* 93:445–456. doi:[10.1111/j.1095-8312.2007.00909.x](https://doi.org/10.1111/j.1095-8312.2007.00909.x)
- Goodman BA, Isaac JL (2008) Convergent body flattening in a clade of tropical rock-using lizards (Scincidae: Lygosominae). *Biol J Linn Soc Lond* 94:399–411. doi:[10.1111/j.1095-8312.2008.00988.x](https://doi.org/10.1111/j.1095-8312.2008.00988.x)
- Greer AE (1989) The biology and evolution of Australian skinks. Surrey Beatty, Sydney
- Hansen TF (1997) Stabilizing selection and the comparative analysis of adaptation. *Evol Int J Org Evol* 51:1341–1351. doi:[10.2307/2411186](https://doi.org/10.2307/2411186)
- Hansen TF, Orzack SH (2005) Assessing current adaptation and phylogenetic inertia as explanations of trait evolution: the need for controlled comparisons. *Evol Int J Org Evol* 59:2063–2072
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755. doi:[10.1093/bioinformatics/17.8.754](https://doi.org/10.1093/bioinformatics/17.8.754)
- Ingram G, Covacevich JA (1988) Revision of the genus *Lygisaurus* de Vis (Scincidae: Reptilia) in Australia. *Mem Queensl Mus* 25:335–354
- Ingram G, Covacevich JA (1989) Revision of the genus *Carlia* (Reptilia, Scincidae) in Australia and comments on *Carlia bicarinata* of New Guinea. *Mem Queensl Mus* 27:443–490
- Jackson JF (1973) Distribution and population phenetics of the Florida scrub lizard, *Sceloporus woodi*. *Copeia* 1973:746–761. doi:[10.2307/1443075](https://doi.org/10.2307/1443075)
- Jaksic FM, Núñez H (1979) Escaping behavior and morphological correlates in two *Liolaemus* species of central Chile (Lacertilia: Iguanidae). *Oecologia* 42:119–122
- Jaksic FM, Nunez H, Ojeda FP (1980) Body proportions, microhabitat selection, and adaptive radiation of *Liolaemus* lizards in Central Chile. *Oecologia* 45:178–181. doi:[10.1007/BF00346457](https://doi.org/10.1007/BF00346457)
- Kraus F (2007) Taxonomic partitioning within Papuan members of the *Carlia novaeguineae* complex (Squamata : Scincidae). *J Herpetol* 41:410–423. doi:[10.1670/0022-1511\(2007\)41\[410:TPWPMO\]2.0.CO;2](https://doi.org/10.1670/0022-1511(2007)41[410:TPWPMO]2.0.CO;2)
- Langerhans RB, Gifford ME, Joseph EO (2007) Ecological speciation in *Gambusia* fishes. *Evol Int J Org Evol* 61:2056–2074. doi:[10.1111/j.1558-5646.2007.00171.x](https://doi.org/10.1111/j.1558-5646.2007.00171.x)

- Langkilde T, Schwartzkopf L, Alford R (2003) An ethogram for adult male rainbow skinks, *Carlia jarnoldae*. *Herpetol J* 13:141–148
- Langkilde T, Schwarzkoff L, Alford RA (2005) The function of tail displays in male rainbow skinks (*Carlia jarnoldae*). *J Herpetol* 39:325–328. doi:10.1670/0022-1511(2005)039[0325:TFOTDI]2.0.CO;2
- Maddison WP, Maddison DR (2008) Mesquite: a modular system for evolutionary analysis
- Malhotra A, Thorpe RS (1997) Size and shape variation in a Lesser Antillean anole, *Anolis oculatus* (Sauria: Iguanidae) in relation to habitat. *Biol J Linn Soc Lond* 60:53–72
- Marchetti K (1993) Dark habitats and bright birds illustrate the role of the environment in species divergence. *Nature* 362:149–152. doi:10.1038/362149a0
- Martins EP (1994) Estimating the rate of phenotypic evolution from comparative data. *Am Nat* 144:193–209. doi:10.1086/285670
- Martins EP (2004) COMPARE, version 4.6b. Computer programs for the statistical analysis of comparative data. Distributed by the author at <http://compare.bio.indiana.edu/>. Department of Biology, Indiana University, Bloomington
- Martins EP, Hansen TF (1997) Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am Nat* 149:646–667. doi:10.1086/286013
- Martins EP, Diniz JAF, Housworth EA (2002) Adaptive constraints and the phylogenetic comparative method: a computer simulation test. *Evol Int J Org Evol* 56:1–13
- Martins EP, Labra A, Halloy M, Thompson JT (2004) Large-scale patterns of signal evolution: an interspecific study of *Liolaemus* lizard headbob displays. *Anim Behav* 68:453–463. doi:10.1016/j.anbehav.2003.08.026
- Mayr E (1947) Ecological factors in speciation. *Evol Int J Org Evol* 1:263–288. doi:10.2307/2405327
- Miles DB (1994) Covariation between morphology and locomotory performance in sceloporine lizards. In: Vitt LJ, Pianka ER (eds) *Lizard ecology*. Princeton University Press, Princeton New Jersey, pp 207–235
- Muller HJ (1942) Isolating mechanisms, evolution, and temperature. *Biol Symp* 6:71–125
- Ord TJ, Stuart-Fox D (2006) Ornament evolution in dragon lizards: multiple gains and widespread losses reveal a complex history of evolutionary change. *J Evol Biol* 19:797–808. doi:10.1111/j.1420-9101.2005.01050.x
- Pianka ER (1969) Sympatry of desert lizards (*Ctenotus*) in Western Australia. *Ecology* 50:1012–1030. doi:10.2307/1936893
- Revell LJ, Johnson MA, Schulte JA, Kolbe JJ, Losos JB (2007) A phylogenetic test for adaptive convergence in rock-dwelling lizards. *Evol Int J Org Evol* 61:2898–2912. doi:10.1111/j.1558-5646.2007.00225.x
- Reznick DN, Butler MJ, Rodd FH, Ross P (1996) Life-history evolution in guppies (*Poecilia reticulata*). 6. Differential mortality as a mechanism for natural selection. *Evol Int J Org Evol* 50:1651–1660. doi:10.2307/2410901
- Sanderson MJ (2002) Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Mol Biol Evol* 19:101–109
- Sanderson M (2004) r8s. Section of ecology and evolution. University of California, Davis
- Schluter D (2000) *The ecology of adaptive radiation*. Oxford University Press, Oxford
- Schneider CJ, Smith TB, Larison B, Moritz C (1999) A test of alternative models of diversification in tropical rainforests: ecological gradients vs. rainforest refugia. *Proc Natl Acad Sci* 96:13869–13873. doi:10.1073/pnas.96.24.13869
- Shine R (1980) Ecology of eastern Australian whipsnakes of the genus *Demansia*. *J Herpetol* 14:381–389. doi:10.2307/1563694
- Sillman AJ, Govardovskii VI, Rohlich P, Southard JA, Loew ER (1997) The photoreceptors and visual pigments of the garter snake (*Thamnophis sirtalis*): a microspectrophotometric, scanning electron microscopic and immunocytochemical study. *J Comparative Physiol Sensory Neural Behav Physiol* 181:89–101
- Smith TB, Schneider CJ, Holder K (2001) Refugial isolation versus ecological gradients. *Genetica* 112:383–398. doi:10.1023/A:1013312510860
- Storr GM (1974) The genus *Carlia* (Lacertilia, Scincidae) in Western Australia and the Northern Territory. *Rec West Aust Mus* 3:151–165
- Stuart-Fox DM, Ord TJ (2004) Sexual selection, natural selection and the evolution of dimorphic coloration and ornamentation in agamid lizards. *Proc R Soc Lond B Biol Sci* 271:2249–2255. doi:10.1098/rspb.2004.2802
- Van Damme R, Vanhooydonck B (2001) Origins of interspecific variation in lizard sprint capacity. *Funct Ecol* 15:186–202. doi:10.1046/j.1365-2435.2001.00513.x
- Vitt LJ (1991) An introduction to the ecology of Cerrado lizards. *J Herpetol* 25:79–90. doi:10.2307/1564798

- Vitt LJ, Caldwell JP, Zani PA, Titus TA (1997) The role of habitat shift in the evolution of Lizard morphology: evidence from tropical Tropicidurus. Proc Natl Acad Sci USA 94:3828–3832. doi:[10.1073/pnas.94.8.3828](https://doi.org/10.1073/pnas.94.8.3828)
- Wainwright PC, Reilly SM (1994) Ecological morphology: integrative organismal biology. University of Chicago Press, Chicago
- Whittier JM (1993) Ecological notes on *Carlia rostralis* in rainforest and associated habitat in the southern Wet Tropics. Mem Queensl Mus 34:125–129
- Zug GR (2004) Systematics of the *Carlia "fusca"* lizards (Squamata: Scincidae) of New Guinea and nearby islands. Bishop Museum Press, Honolulu