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SHORT COMMUNICATION

Species with a chemical defence, but not chemical offence, live longer

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Abstract

Evolutionary hypotheses for ageing generally predict that delayed senescence should evolve in organisms that experience lower extrinsic mortality. Thus, one might expect species that are highly toxic or venomous (i.e. chemically protected) will have longer lifespans than related species that are not likewise protected. This remarkable relationship has been suggested to occur in amphibians and snakes. First, we show that chemical protection is highly conserved in several lineages of amphibians and snakes. Therefore, accounting for phylogenetic autocorrelation is critical when conservatively testing evolutionary hypotheses because species may possess similar longevities and defensive attributes simply through shared ancestry. Herein, we compare maximum longevity of chemically protected and non-protected species, controlling for potential nonindependence of traits among species using recently available phylogenies. Our analyses confirm that longevity is positively correlated with body size in both groups which is consistent with life-history theory. We also show that maximum lifespan was positively associated with chemical protection in amphibian species but not in snakes. Chemical protection is defensive in amphibians, but primarily offensive (involved in prey capture) in snakes. Thus, we find that although chemical defence in amphibians favours long life, there is no evidence that chemical offence in snakes does the same.

Introduction

Several general evolutionary hypotheses have been proposed to explain why organisms tend to experience higher mortality as they age and, consequently, why there is such wide variation in longevity among species (Medawar, 1952; Williams, 1957; Kirkwood, 1977, 1996; Kirkwood & Holliday, 1979). These hypotheses (summarized in Blanco & Sherman, 2005) are united in their prediction of a positive relationship between the rate of 'extrinsic' mortality and the rate of senescence (but for important qualifications see Abrams, 1993; Ricklefs, 1998; Williams & Day, 2003). This relationship was examined by Blanco & Sherman (2005) who compared the maximum recorded longevity in captivity of chemically protected and unprotected species of fish, amphibians and snakes. Their cross-species comparison found a positive relationship between maximum longevity and possession of chemical protection in all groups examined. Assuming that chemically protected species experience lower extrinsic mortality on average than unprotected species, then this result supports the broad predictions of the evolutionary hypotheses for ageing. However, key differences may exist in the extent to which purely defensive chemicals (e.g. skin toxins) translate to reductions in extrinsic mortality in the field compared with those traits where the primary function is prey capture (e.g. snake venom).

An important limitation of Blanco & Sherman (2005), recognized by the authors, is that their comparisons did

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not completely account for the potential lack of independence of characteristics among phylogenetically related species. This omission is important because related species may possess similar traits not only through independent realizations of the same causal process, but also because of their shared evolutionary history (Harvey & Pagel, 1991). The assumption of data independence is fundamental in statistical tests, and its contravention necessarily calls into question any results. Although there remains discussion over whether there is ever sufficient phylogenetic inertia to require 'correction' (Reeve & Sherman, 2001), the fact that it may exist suggests that we should consider controlling for it, especially when conservatively testing evolutionary hypotheses. At minimum, it is helpful to compare tests of evolutionary hypotheses with and without controls for phylogenetic autocorrelation. Blanco & Sherman (2005) partially circumvented the problem of phylogenetic dependence by reanalysing their data using characters classified at the genus level. However, this approach necessarily overlooks intrageneric variation and continues to assume independence of characters at higher taxonomic levels (e.g. at the family and subfamily level). Advances in comparative biology and recently available phylogenies now allow us to conduct appropriate phylogenetically controlled analyses. Such phylogenetic controls are critical in cases of high phylogenetic dependence (see Swiderski, 2001 and references therein, Freckleton *et al.*, 2002), and our reanalysis therefore provides the first truly rigorous test of the relationship between longevity and possession of chemical protection in amphibians and snakes.

Materials and methods

Longevity, size and chemical protection data were obtained from supplementary tables generously made available by Blanco & Sherman (2005). Snout-vent length was used for the amphibian size data, whereas total length was used for snakes because of data availability. Data for an additional seven snake species were collected from Ernst & Ernst (2012). We reclassified three snake species considered nonchemically protected by Blanco & Sherman (2005) as chemically protected because Weinstein *et al.* (2011) indicated that they possessed potent venom: *Enhydris chinensis* (murine intravenous LD_{50} = 2.05 mg kg^{-1}), *Spalerosophis diadema* (murine intravenous LD_{50} = 2.75 mg kg^{-1}) and *Thamnophis elegans* (murine intraperitoneal LD_{50} 13.85 mg kg^{-1}). In our analyses, we used recently published phylogenies (Amphibia: Pyron & Wiens, 2011; Colubroidea: Pyron *et al.*, 2011) which represent the most comprehensive phylogenies for these groups to date. Analyses were restricted to those species where body size, longevity and phylogenetic data were both available (amphibians: n = 106, snakes: n = 102).

To evaluate the extent of phylogenetic dependence of species-level traits (i.e. longevity, maximum body size and possession of chemical protection), we computed Pagel's λ (Pagel, 1992; Freckleton *et al.*, 2002) through maximum likelihood optimization using the geiger package (Harmon *et al.*, 2009) in R (R Development Core Team, 2012). We then compared the negative log likelihood from the optimized λ value with that from a tree without phylogenetic signal (i.e. $\lambda = 0$) using a likelihood ratio test to determine statistical significance. The relationship between chemical protection and longevity was examined using both phylogenetic generalized least squares (PGLS) and generalized estimating equation (GEE) approaches (Grafen, 1989; Paradis & Claude, 2002). PGLS has low Type I error rates and is suggested to be one of the most robust methods currently available to examine evolutionary trends (Laurin, 2010). Maximum body size (i.e. length) was included as a factor in our analyses because Blanco & Sherman (2005) suggested a positive effect of body size on longevity in these groups, and mass data were not available. We have not reanalysed the fish data from Blanco & Sherman (2005) because a suitable phylogeny is not yet available. To illustrate the importance of controlling for phylogenetic dependence, we also analysed our data by fitting a general linear model (GLM) that did not control for such dependencies. Models were fitted in R using the ape (Paradis *et al.*, 2009) and nlme (Pinheiro *et al.*, 2012) packages.

Results

As expected, chemical protection showed strong phylogenetic signal in both amphibians and snakes (Table 1, Fig. 1) indicating that the possession of chemical protection is not independent among related taxa. Similarly, there was strong phylogenetic signal in body size, yet intriguingly no phylogenetic signal was detected in longevity (Table 1). In amphibians, both maximum body size and possession of chemical protection had a significant positive relationship with maximum longevity. However, maximum body size, but not chemical protection, had a

Table 1 The strength of phylogenetic signal estimated for longevity, maximum body size, and chemical protection in both amphibians (n = 106) and advanced snakes (n = 102) as indicated by Pagel's λ values estimated through maximum likelihood (ML) optimization. P-values indicate that the ML λ is significantly different from 0, suggesting that trait values do not vary independently between species, but instead exhibit phylogenetic autocorrelation.

	Amphibians		Snakes	
	λ	P-value	λ	P-value
Longevity	<0.001	0.999	<0.001	0.984
Size	0.833	0.016	0.918	0.015
Chemical protection	1	0.003	0.956	<0.001

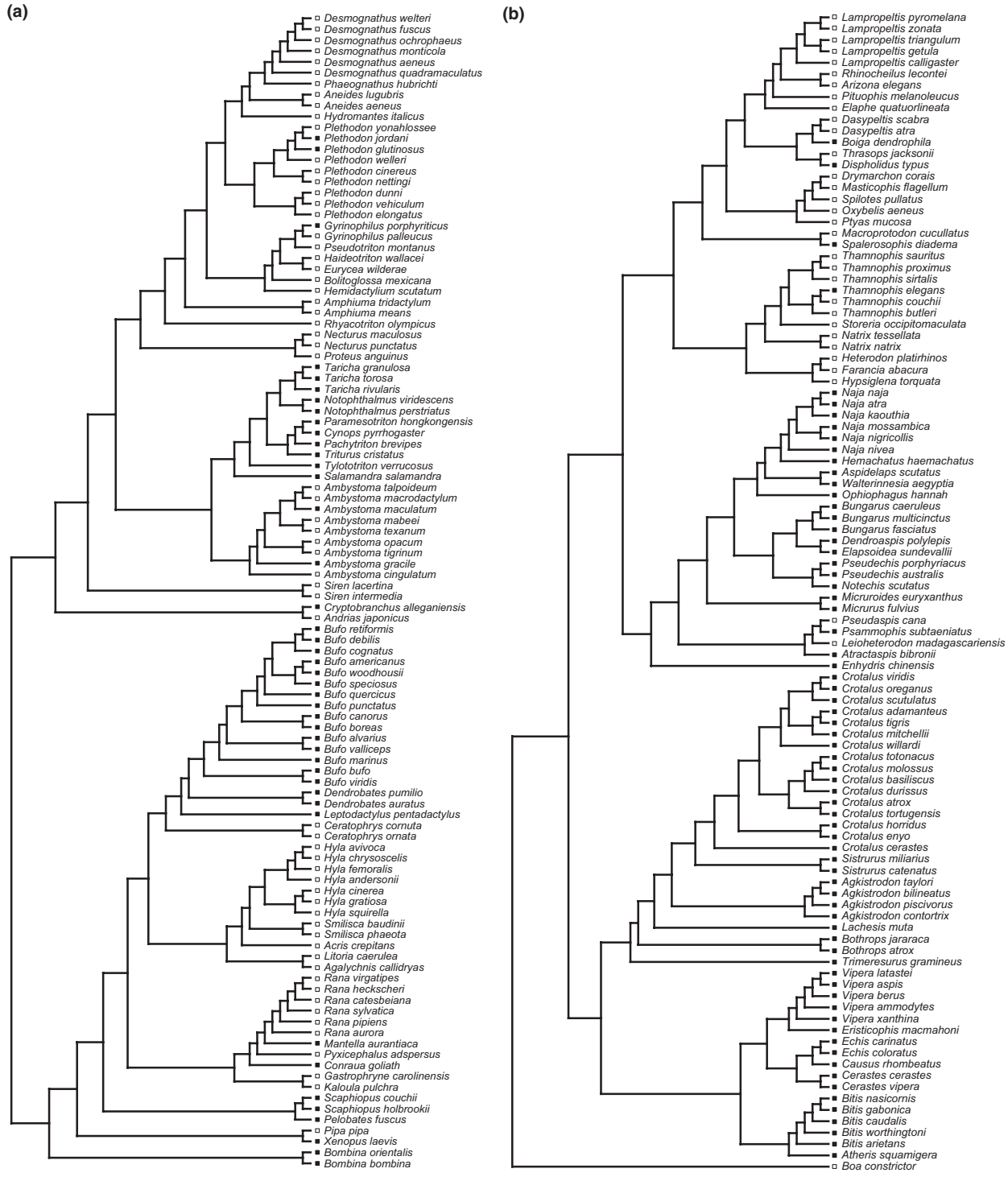

Fig. 1 Cladograms showing evolutionary relationships between (a) amphibians and (b) snakes. In each cladogram, a filled square on the terminal branch indicates chemical protection. Classifications largely from Blanco & Sherman (2005) but see main text for exceptions.

significant positive relationship with longevity in snakes (Tables 2, S1). Both the GEE and PGLS approaches generated similar results (Table 2). In contrast, failing to control for phylogenetic dependence greatly overestimated the significance of these relationships in both amphibians and snakes (Tables 2, S1).

Table 2 Results from phylogenetic generalized least squares (PGLS), generalized estimating equation (GEE) and general linear model (GLM) without phylogenetic control examining the effects of maximum body size and being chemically protected on longevity in amphibians (n = 106) and advanced snakes (n = 102). Body size and longevity were continuous variables, whereas chemical protection was categorical (0 = unprotected, 1 = protected). For PGLS and GLM models, results from analyses with Type I (sequential) sum of squares are shown, but qualitatively similar results were generated with Type III (adjusted) sum of squares. d.f.P = phylogenetic degrees of freedom, for details see (Paradis *et al.*, 2009).

		PGLS – Grafen			GEE			GLM		
Term	d.f.	*Parameter Est.*	*t*	*P*-value	d.f.P	*t*	*P*-value	d.f.	*F*	*P*-value
Amphibians										
Size	1	0.22	261.46	<0.001	1	7.94	<0.001	1	52.04	<0.001
Chemical protection	1	3.82	52.04	0.006	1	15.9	<0.001	1	7.91	0.006
Error	103				16.5			103		
Snakes										
Size	1	0.03	4.02	<0.001	1	7.57	<0.001	1	15.96	<0.001
Chemical protection	1	0.08	0.04	0.971	1	0.4	0.69	1	3.51	0.064
Error	99				21.99			99		

Discussion

Amphibian longevity was associated positively with the possession of chemical protection, and body size was positively associated with longevity in both amphibians and snakes, supporting the wider predictions of senescence theory and consistent with the results of Blanco & Sherman (2005). Indeed, the strong positive effect of body size on longevity may be a result of larger animals suffering reduced extrinsic mortality (see also Wilkinson & South, 2002). Nevertheless, we found no association between longevity and the possession of potent venom in snakes, which contrasts directly with the earlier interpretation of Blanco & Sherman (2005).

The lack of a relationship between possession of potent venom and longevity in snakes most likely results from the dual purpose of venom in both predation and defence. Defensive bites vary greatly in venom load (indeed, some venomous snakes may bite without venom), whereas predatory bites are metered according to prey size and species (Hayes *et al.*, 2002). Furthermore, recent comparative analyses support the generally accepted hypothesis that snake venom evolved primarily as a predatory, offensive weapon (Barlow *et al.*, 2009), making it a qualitatively different trait to the chemical defence in amphibians. We were not able to find any examples of amphibians where the primary use of their toxins was prey capture. Alternative explanations for the lack of a positive association include the possibility that the dichotomous classification of snakes as venomous vs. nonvenomous poorly reflects the trait's continuous nature. Of course, it may also arise as a Type II statistical error, but our test has reasonable power and included data from 102 species of snakes from 54 genera and four families. We must also acknowledge the possibility of greater error in the measurement of maximum lifespan in snakes, as compared to amphibians. Snakes are relatively long-lived when compared to amphibians, and their longevity records from zoos occasionally came from individuals that were wild-caught as adults of an unknown age, were still alive at the time age was reported, or both (see also: Moorad *et al.*, 2012).

In summary, our findings demonstrate the importance of phylogenetic methods in reducing Type I errors when conducting comparative studies and showcase the power of such techniques to examine evolutionary explanations for phenomena such as senescence. Our findings are consistent with a primarily offensive role for snake venom, which is not expected to reduce extrinsic mortality in the same way as an anti-predator trait, and a defensive role for amphibian toxins that alleviates that extrinsic mortality. Furthermore, our work illustrates that elucidating evolutionary patterns in longevity relies on a well-developed understanding of the realized ecological function of traits assumed to reduce extrinsic mortality.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Amphibian phylogeny used in our analyses, reduced from Pyron *et al.* (2011).

Figure S2 Snake phylogeny used in our analyses, reduced from Pyron & Wiens (2011).

Figure S3 Mean (±SE) maximum longevities for chemically protected and unprotected species (as classified by Blanco & Sherman, 2005) within families of amphibians (left), and snakes (right).

Table S1 Results from Phylogenetic Generalized Least Squares (PGLS), examining the effects of maximum body size and being chemically defended on longevity in amphibians (n = 106) and advanced snakes (n = 102) when the correlation structure based on a Brownian, *Ornstein-Uhlenbeck*, or Grafen *model.*

Table S2 Raw data for maximum longevity, maximum size (body length), and presence/absence of chemical protection for amphibian species. See main text for data sources.

Table S3 Raw data for maximum longevity, maximum size (body length), and presence/absence of chemical protection for snake species. See main text for data sources.

Data deposited at Dryad: doi:10.5061/dryad.bg49s

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