Individual-level diet variation in four species of Brazilian frogs

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Summary

1. Many natural populations exploiting a wide range of resources are actually composed of relatively specialized individuals.

2. This interindividual variation is thought to be a consequence of the invasion of 'empty' niches in depauperate communities, generally in temperate regions. If individual niches are constrained by functional trade-offs, the expansion of the population niche is only achieved by an increase in interindividual variation, consistent with the 'niche variation hypothesis'.

3. According to this hypothesis, we should not expect interindividual variation in species belonging to highly diverse, packed communities.

4. In the present study, we measured the degree of interindividual diet variation in four species of frogs of the highly diverse Brazilian Cerrado, using both gut contents and $\delta^{13}C$ stable isotopes.

5. We found evidence of significant diet variation in the four species, indicating that this phenomenon is not restricted to depauperate communities in temperate regions.

6. The lack of correlations between the frogs' morphology and diet indicate that trade-offs do not depend on the morphological characters measured here and are probably not biomechanical. The nature of the trade-offs remains unknown, but are likely to be cognitive or physiological.

7. Finally, we found a positive correlation between the population niche width and the degree of diet variation, but a null model showed that this correlation can be generated by individuals sampling randomly from a common set of resources. Therefore, albeit consistent with, our results cannot be taken as evidence in favour of the niche variation hypothesis.

Key-words: carbon stable isotopes, individual specialization, niche variation, Niche Variation Hypothesis

Introduction

Many natural populations exploiting a wide variety of resources are actually composed of relatively specialist individuals (West 1986; Werner & Sherry 1987; Svanbäck & Bolnick 2007; Araújo *et al.* 2008). This 'individual specialization', which has been documented in more than 100 taxa (Bolnick *et al.* 2003), may have important ecological and evolutionary implications. For example, several models of demographic stochasticity and population dynamics predict that ecologically

variable populations have more stable dynamics (Lomnicki 1988; Kendall & Fox 2002, 2003; Fox 2005), a prediction recently confirmed empirically in experimental populations of flour beetles (Agashe, in press). Additionally, if individuals use only a subset of the population niche, competitive interactions will be frequency dependent and will favour rare strategies (Wilson & Turelli 1986; Bolnick 2001). This in turn may drive disruptive selection (Bolnick 2004; Pfennig, Rice & Martin 2007; Bolnick & Lau 2008), which may increase the population genetic and phenotypic variance (Roughgarden, 1972) and cause evolutionary divergence (Dieckmann & Doebeli, 1999; Doebeli *et al.* 2007).

While most studies published so far have only been able to document the presence of individual specialization in natural populations (Bolnick *et al.* 2003), available indices that actually

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measure the *degree* of individual specialization open the possibility of testing some predictions (Bolnick *et al.* 2002). For example, the occurrence of individual specialization is generally accredited to the invasion of 'empty' niches following ecological release in depauperate communities (Ebenman & Nilsson 1982; Robinson *et al.* 1993; Smith & Skúlason 1996). During competitive release, although the population niche expands, individual niches may be constrained by functional trade-offs (Smith, 1987; Ehlinger & Wilson 1988; Schluter 1995; Robinson 2000). Consequently, the population niche expansion arises by increased among-individual variation, rather than increased individual niche width. Contrasting this view, some recent studies have reported strong degrees of individual-level diet variation in several tropical species, including frogs (Araújo *et al.* 2007b), a hunting-wasp (Araújo & Gonzaga 2007) and a marsupial (Martins *et al.* 2008), all belonging to highly diverse communities in which no ecological release is expected. How general this pattern is in tropical species and communities is still unknown.

In line with the idea that niche expansion may cause interindividual variation, Bolnick *et al.* (2007) demonstrated the existence of a positive relationship between the width of population niches and the degree of interindividual diet variation in several disparate taxa (frogs, fishes, snails, and lizards), corroborating Van Valen's (1965) 'niche variation hypothesis' (NVH). In the present study, we measured the degree of individual specialization in four additional species of frogs inhabiting a region with high diversity of frogs (Giaretta *et al.* 2008) in the Brazilian Cerrado (extending a previous study of four sympatric Cerrado frog species; Araújo *et al.* 2007b). By doing this we have the opportunity to (i) further test the hypothesis that tropical species should show negligible degree of individual specialization, and (ii) that populations with wider niches show higher degrees of individual specialization (NVH). Finally, we (iii) investigated the presence of correlations between the frogs' morphology and diet. Such correlations would be indicative of the presence of biomechanical trade-offs, which would offer a mechanistic explanation for diet variation.

Materials and methods

STUDY AREA

We analysed the stomach contents and stable carbon isotopes of muscle tissue of four species of frogs from a savannah formation in south-eastern Brazil locally known as Cerrado (Oliveira & Marquis 2002). There is marked seasonality in the area, with a wet/warm season (henceforth 'wet season') from September to March and a dry/mild season (henceforth 'dry season') from April to August (Rosa, Lima & Assunção 1991). Specimens of four species [*Physalaemus cuvieri* Fitzinger, 1826, *Eupemphix nattereri* Steindachner, 1863, *Chiasmocleis albopunctata* (Boettger, 1885), and *Elachistocleis bicolor* (Guérin-Meneville, 1838); *n* = 60, 65, 51, and 54 individuals respectively] were obtained from the collection of the Museu de Biodiversidade do Cerrado of the Universidade Federal de Uberlândia (MBC-UFU). Specimens were collected in the municipality of Uberlândia (18°55′S, 48°17′W, 850 m), in the state of Minas Gerais,

south-eastern Brazil, at the Clube de Caça e Pesca Itororó de Uberlândia (*P. cuvieri*, *E. nattereri*, and *E. bicolor*), and at the Estação Ecológica do Panga (*C. albopunctata*). Details on the study area are provided in Araújo *et al.* (2007b). Frogs were collected weekly in the wet season and once every two weeks in the dry season, for a period of 2 years. Specimens of *P. cuvieri* were collected from October 1999 to November 2000; *E. nattereri* from October 1999 to October 2001; *C. albopunctata* from November 2000 to October 2001; and *E. bicolor* from October 1999 to March 2001. Frogs were immediately killed upon collection, preserved in 5% formalin and later transferred to 70% ethanol.

DATA COLLECTION

Morphology

Five measurements were taken from each specimen with digital calipers (nearest 0·01 mm) always by the same person (M.S.A.): snout-vent length (SVL), mouth width, lower jaw length, head length, and eye–nostril distance. We did not measure the mass of individuals, because preservative absorption was likely to bias our results.

Diet data

Diets were quantified by the analysis of stomach contents of the preserved specimens, which were dissected to obtain stomach contents. Prey items were counted, and identified to the lowest taxonomic level possible (Order and Family, in most cases). We recognize that by lumping prey into such broad taxonomic groups, we are ignoring substantial variation in resources, which may in turn lead to an underestimation of the degree of individual specialization (Bolnick *et al.* 2002). Our results therefore may be seen as a conservative estimate of the degree of individual specialization in these species. Upon dissection, individuals were sexed by examination of gonads and classified as juveniles or adults (see Araújo *et al.* 2007b for details).

Stable isotopes

We measured carbon stable isotopes from the preserved frogs. Araújo *et al.* (2007a) measured the carbon isotopes of the prey consumed by four other frog species that inhabit the same areas as the species studied here and were collected at the same time period. The prey taxa found in the present study (see Results) were the same found by Araújo *et al.* (2007a). We therefore assigned the same $\delta^{13}C$ signatures reported by those authors to the prey taxa found here.

Carbon isotopic signatures of animal tissues can be altered by ethanol and formalin preservation (Kaehler & Pakhomov 2001; Sweeting, Polunin & Jennings, 2004). However, since we are interested in estimating the variance among individual isotopic ratios (see below, Data analyses) and all our samples were subject to the same preservation conditions, preservation should not be a problem in our study.

The processing of samples follows Araújo *et al.* (2007a). The abundances of ^{13}C and ^{12}C were determined at the Centro de Energia Nuclear na Agricultura of the Universidade de São Paulo (CENA/USP) in Piracicaba. Samples were analysed in a Micromass 602E mass spectrometer (Finnegan Mat, Bremen, Germany) fitted with double inlet and collector systems. Organic standards (BBOT) were run every 12 samples and their mean ± standard error was –26·9 ± 0·06. Additionally, five randomly picked samples were duplicated. Experimental precision was measured as the mean ± standard error of the repeatability of duplicates and was 0·1 ± 0·02‰. The $^{13}C/^{12}C$

compositions are reported using conventional delta notation, showing differences between the observed concentration and that of Pee Dee Belemnite (PDB).

Due to the small sample sizes in the dry season (*P. cuvieri*, $n = 2$ individuals; *E. nattereri*, $n = 10$; *C. albopunctata*, $n = 1$; and *E. bicolor*, $n = 2$) we analysed only the individuals collected in the wet season (when diet variation tends to be stronger; Araújo *et al.* 2007b). In the analyses of diet data, only the individuals having any content in their stomachs were analysed, which explains the differences in sample sizes between diet and isotope analyses.

Diet data

Individual-level diet variation may be confounded with other forms of intrapopulation variation if individuals of different age classes or different sexes are analysed. Therefore, before investigating individual specialization we tested for ontogenetic and sex-related diet differences. In order to investigate ontogenetic diet shifts, we used Schoener's (1968) proportional similarity index (*PS*),

$$PS_{ij} = 1 - 0.5\sum_k |p_{ik} - p_{jk}|,$$

in which p_{ik} and p_{jk} are the proportions of prey category k in the diets of i and j. PS_{ij} is the overlap between the diets of i and j, varying from 0 (no overlap) to 1 (total overlap). We tested for age and sex-related differences in diet using the *PS* index. In the comparisons between sexes, only adults were analysed. For the sake of statistical power, when no ontogenetic and/or sex-related differences in diets were found, we pooled individuals of different age-classes and/or sexes in the analyses of individual specialization.

In order to measure individual-level diet variation, we used the proposed adaptation of the proportional similarity index, PS_i, which measures the overlap between an individual i's diet and the population diet. Details on this index can be found in Bolnick *et al.* (2002). Briefly, the PS_i values of all individuals in the population can be calculated and summarized as a population-wide measure of individual specialization, which is the average of PS_i values, *IS* (Bolnick *et al.* 2002). *IS* varies from near 0 (maximum individual specialization) to 1 (no individual specialization). In order to make this measure more intuitive, we use $V = 1 - IS$, so that higher values now indicate higher individual specialization.

The calculation of all indices was performed in INDSPEC 1·0, a program to calculate indices of individual specialization (Bolnick *et al.* 2002). In INDSPEC 1·0, the proportion of diet categories in the population diet can be calculated either by adding up the prey counts of all individuals for each resource and dividing it by the total count of prey for the population, or by converting the counts of each individual to proportions and averaging them across all individuals for each resource (Bolnick *et al.* 2002). We used the latter method, whereas Bolnick *et al.* (2007) used the former, which explains the slight differences between the measures of *V* in Bolnick *et al.* (2007) and in the present study (see Diet variation vs. population niche width below). We also used INDSPEC 1·0 to calculate the significance of the *PS* measures between age classes, sexes, and the *V* measures of individual specialization. INDSPEC 1·0 uses a nonparametric Monte Carlo procedure to generate replicate null diet matrices drawn from the population distribution (Bolnick *et al.* 2002), from which *P* values can be computed. The null model relies on the assumption that each prey item in the diet corresponds to an independent feeding event, which we acknowledge is probably untrue in the case of termites and ants. We used 10 000 replicates in Monte Carlo bootstrap simulations to obtain *P* values for these indices.

Stable isotopes

Many studies focusing on individual specialization have relied on gut contents as a source of diet information (Bryan & Larkin 1972; Roughgarden 1974; Robinson *et al.* 1993; Schindler 1997; Fermon & Cibert 1998; Warburton, Retif & Hume 1998; Svanbäck & Bolnick 2007). However, gut contents are a 'snapshot' of an individual's diet and do not necessarily reflect long-term preferences (Warburton *et al.* 1998). This sampling problem may make one believe that individuals are more specialized than they really are, leading to an overestimate of the degree of individual specialization in the population (Bolnick *et al.* 2003). Therefore, in studies using gut-content data, it is desirable to have some measure of temporal consistency in food resource use by individuals (Bolnick *et al.* 2003). Several studies have measured stable isotopes (Fry, Joern & Parker 1978; Gu, Schelske & Hoyer 1997) to infer temporal consistency in the diets of individuals. Due to their slow turnover (Tieszen *et al.* 1983), isotopes will not be subject to the same stochastic sampling effects as gut contents and can be a more reliable way to infer individual temporal consistency in resource use. In fact, the among-individual variation in $\delta^{13}C$ signatures can be interpreted as a measure of individual-level diet variation (Fry *et al.* 1978; Angerbjörn *et al.* 1994; Gu *et al.* 1997; Sweeting, Jennings & Polunin 2005). If the individuals in a given population all have similar diets, they will also show similar isotopic signatures, so that the population isotopic variance will be low. On the other hand, if individuals vary in their isotopic signatures, this can be taken as evidence of long-term interindividual diet variation.

However, using isotopes to estimate diet variation has some drawbacks. If the number of food resources is higher than one can discriminate with isotopes (Phillips & Gregg 2003), isotope variation can underestimate diet variation (Matthews & Mazumder 2004). On the other hand, if resources vary in their isotopic composition in space or time and consumers are sampled in different places and/or times, there will be isotopic variation that cannot be attributed to diet variation. In the same line, variation in fractionation among individual consumers and isotopic variation within food resources themselves may also increase variation in consumers (Moore & Semmens 2008). Finally, for a given level of diet variation, populations using more isotopically variable prey (e.g. –34, –32, –30, –28, –26‰) will show higher isotopic variances than populations using less variable prey (e.g. –31, –30, –29, –28, –27‰; Matthews & Mazumder 2004). Consequently, measures of population isotopic variance per se can be a misleading guide to diet variation if the prey isotopic variance is not taken into account. These caveats, although not invalidating the use of stable isotopes in studies of individual specialization, underscore the necessity of using other sources of diet information (e.g. gut contents) as complementary approaches.

Bearing those caveats in mind, we used a method that converts a measure of $\delta^{13}C$ variance in consumers into an estimate of the *V* index of individual specialization (Araújo *et al.* 2007a). This method uses empirical diet data of consumers and isotope data of prey to generate an expected relationship between the $\delta^{13}C$ variance and the *V* measure of diet variation. We then use this relationship to convert the empirical variance in the isotopes of consumers into an estimate of *V*. Readers are referred to Araújo *et al.* (2007a) for the details on

the method. The parameters used in the model were the population diet (estimated empirically from gut contents), the prey $\delta^{13}C$ signatures and dry masses (taken from Araújo *et al.* 2007a) and the $\delta^{13}C$ variance of consumers (also estimated empirically). Simulations were run in the program VARISO 1·0 (Araújo *et al.* 2007a).

Morphology vs. diet

We tested for the correlation between morphology and diet. Such correlations would be indicative of the presence of biomechanical trade-offs, which would offer a mechanistic explanation for diet variation. In order to test this correlation, we took an approach that relates body shape to interindividual diet overlap. Within each species, we first did a principal component analysis (PCA) on the five log-transformed morphological measurements. We then calculated a matrix of pairwise Euclidean morphological distances based on the PC2–PC5 scores (interpreted as body shape) among all individuals. Next, we calculated a matrix of pairwise diet overlap among individuals, using the *PS* index, in which p_{ik} and p_{jk} are the proportions of prey category *k* in individual *i*'s and *j*'s diet, respectively. If there were an effect of functional morphology on diet, we would expect that morphologically similar individuals (small Euclidean distances) also show similar diets (high diet overlap), and vice-versa. If this were true, we would expect a negative correlation between the matrices of morphological distance and diet overlap. We tested the correlation between matrices with a simple Mantel test with 10 000 simulations. The PCAs were performed in SYSTAT 11 and the Mantel tests were carried out using the software POPTOOLS 2·6·9 (Hood 2005).

Diet variation vs. population niche width

The total niche width of each population (TNW) was quantified using the Shannon–Weaver diversity index, following Roughgarden (1979). This index will yield a value of zero when the entire population uses only a single category of prey, increasing with both the number of prey categories and the evenness with which they are used. We then took the data for the other four species of frogs reported in Bolnick *et al.* (2007) – *Leptodactylus fuscus*, *Leptodactylus* sp., *Proceratophrys* sp., and *Ischnocnema penaxavantinho* (Giaretta, Toffoli & Oliveira 2007); note that in Bolnick *et al.* (2007), there are two samples for each frog species (wet and dry season)-combined with our data and regressed *V* on TNW. A significant positive slope would confirm a positive relationship between TNW and *V*. Following Bolnick *et al.* (2007), we tested whether this positive slope could be accounted for by a null model involving only stochastic sampling. We used a resampling procedure to recreate this artefact as a null expectation for the relationship between population niche width and among-individual diet variation. For each sample, we took the population niche (the proportions of each prey category in the population diet) and let each individual observed to have consumed some number *n* of prey items to randomly sample *n* items from the population diet frequencies via multinomial sampling. The null degree of diet variation (*V*) was calculated once all individuals were assigned random diets. For each sample, we carried out 10 000 such resampling estimates. We then regressed the mean resampled *V* against the observed TNW to evaluate the null hypothesis that limited individual diet data also generate a positive relationship between these measures. The resampling procedures were carried out in INDSPEC 1·0 and the regression analyses were performed in SYSTAT 11. The NVH is only supported if the empirical slope of *V* as a function of TNW is significantly steeper than the null slope.

Results

DIET DATA

The frogs consumed 21 prey categories including several orders of insects and arachnids, with a clear prevalence of Isoptera and Formicidae in the diets (Fig. 1; see Table S1 for the raw data). *P. cuvieri* had the most and *E. bicolor* the least diverse diets (Fig. 1). We found no evidence of ontogenetic or sex-related differences in diets (Table 1), although there was a marginally significant difference between the diets of males and females in *E. nattereri* (Table 1). Despite the relatively
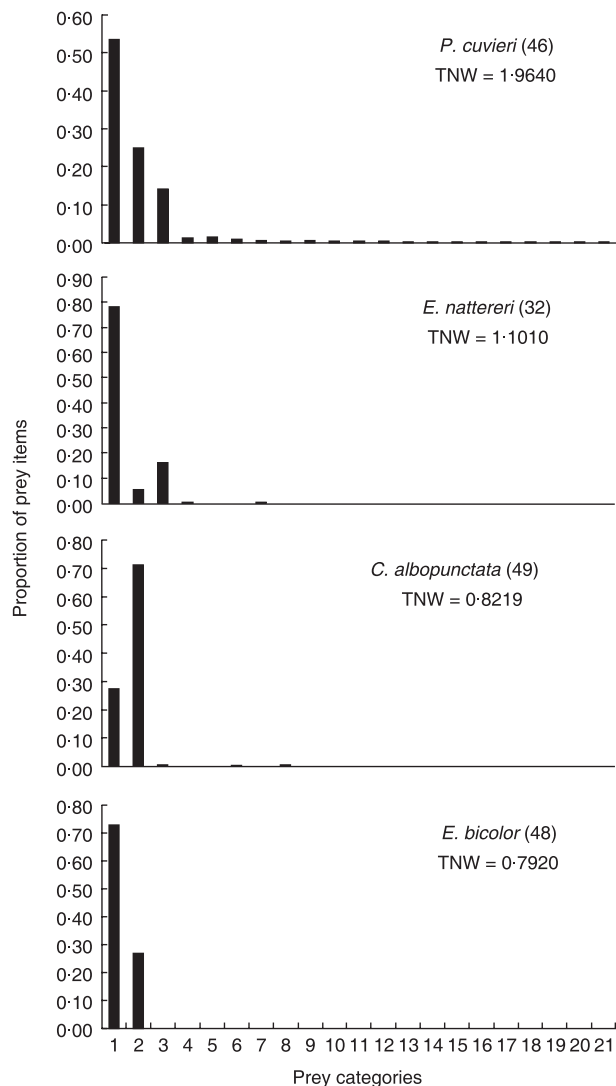
Fig. 1. Diet composition of four frogs of the Brazilian Cerrado, expressed as the proportion of the number of items found in stomachs, and Roughgarden's (1979) measure of population TNW. Resource categories from left to right are: 1, Isoptera (workers and soldiers); 2, Formicidae; 3, Isoptera (alates); 4, Seeds; 5, Araneae; 6, Coleoptera; 7, Insect larvae; 8, Gastropoda; 9, Auchenorrincha; 10, Diptera; 11, Orthoptera; 12, Blattodea; 13, Acari; 14, Collembola; 15, Heteroptera; 16, Hymenoptera (non–Formicidae); 17, Lepidoptera (adults); 18, Isopoda; 19, Opiliones; 20, Mantodea; 21, Oligochaeta. Numbers in parenthesis are the number of analysed frogs.

Table 1. Schoener's (1968) proportional similarity index (*PS*) between the diets of different sexes and age classes (juveniles vs. adults) of four species of Brazilian frogs (*Physalaemus cuvieri*, *Eupemphix nattereri*, *Chiasmocleis albopunctata*, and *Elachistocleis bicolor*). Only adults were used in the comparisons between sexes. *P* values were obtained in Monte Carlo bootstraps (10 000 simulations)

Species	Age classes			Sexes		
	PS	*P*	*n*	PS	*P*	*n*
*Physalaemus cuvieri**	–	–	–	0·8032	0·8714	45
Eupemphix nattereri	0·6861	0·7755	32	0·1924	0·0689	13
Chiasmocleis albopunctata	0·9501	0·9659	49	0·9324	0·7717	45
Elachistocleis bicolor	0·5735	0·9920	48	0·7480	0·2495	43

n, number of frogs. *The small number of juveniles (*n* = 2) prevented the comparison between age classes.

Table 2. The *V* measure of individual specialization in the diets of four species of Brazilian frogs (*Physalaemus cuvieri*, *Eupemphix nattereri*, *Chiasmocleis albopunctata*, and *Elachistocleis bicolor*). Individual specialization was measured using both gut contents and $\delta^{13}C$ stable isotopes. V_{emp}: empirical *V* index of individual specialization based on gut-content data; $Var\delta^{13}C$: empirically estimated variances of individual $\delta^{13}C$ signatures; V_{exp}: expected value of the *V* index based on isotope data (see Methods). V_{emp} values were tested against null distributions generated with Monte Carlo bootstraps (10 000 simulations); **$P < 0.01$; ****$P < 0.0001$

Species	Gut contents		$\delta^{13}C$ stable isotopes		
	V_{emp}	*n*	$Var\delta^{13}C$	V_{exp}	*n*
Physalaemus cuvieri	0·6843****	46	4·6628	0·5820	58
Eupemphix nattereri	0·4976****	32	4·7813	0·3428	55
Chiasmocleis albopunctata	0·3426****	49	3·9004	0·1864	43
Elachistocleis bicolor	0·4349**	48	8·7409	0·4957	54

n, sample size.

narrow population niches (Fig. 1), we found evidence of significant individual specialization in all four species (Table 2). *P. cuvieri* presented the highest and *C. albopunctata* the lowest degree of diet variation (Table 2).

STABLE ISOTOPES

Overall, the species showed large variation in individual $\delta^{13}C$ isotopic signatures that spanned the whole range of prey isotopic signatures (Fig. 2), suggesting the presence of diet variation. *C. albopunctata* showed signatures in line with a diet composed mainly of Formicidae ($\delta^{13}C = -22.8$‰; Araújo *et al.* 2007a), corroborating the gut-content data (Fig. 1). However, *E. bicolor*, which had the narrowest niche according to gut contents (Fig. 1), presented a relatively high variation in isotopes (Fig. 2). Most notably, this species showed a rather bimodal distribution of $\delta^{13}C$ signatures, suggesting that individuals either specialize on Formicidae ($\delta^{13}C = -22.8$‰) or Isoptera ($\delta^{13}C = -14.7$‰; Araújo *et al.* 2007a). In all species, some individuals showed isotopic signatures out of the prey range of $\delta^{13}C$ values (Fig. 2). This mismatch between frogs' and prey signatures may be caused by fractionation in the consumers (Vander Zanden & Rasmussen 2001), by the lack of taxonomic resolution of prey (many prey species or families were lumped when estimating $\delta^{13}C$ isotopes; Araújo *et al.* 2007a), or because some prey categories were missed in gut contents. The latter explanation seems unlikely, though, due to the high sample sizes analysed in Araújo *et al.* (2007a).
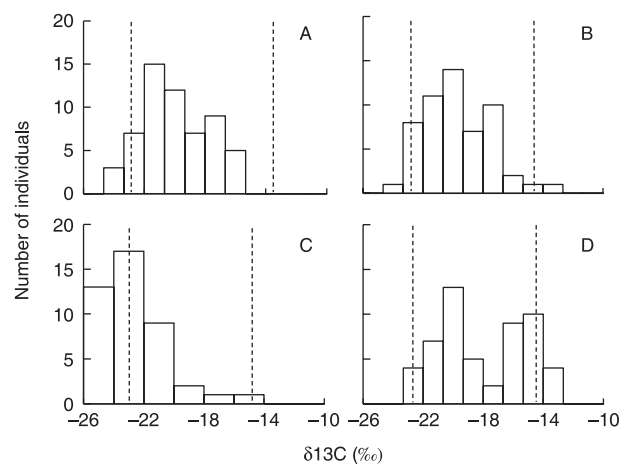
Fig. 2. Histograms of the empirically measured individual $\delta^{13}C$ signatures in four species of Brazilian frogs: A *Physalaemus cuvieri* (*N* = 58); B *Eupemphix nattereri* (*N* = 55); C *Chiasmocleis albopunctata* (*N* = 43); D *Elachistocleis bicolor* (*N* = 54). Dashed lines indicate the range of $\delta^{13}C$ of consumed prey.

C. albopunctata had the smallest and *E. bicolor* the highest variances in $\delta^{13}C$ (Table 2). The isotope-derived *V* measures indicated the existence of individual specialization in all species corroborating the findings based on gut contents (Table 2). With the exception of *E. bicolor*, the isotope-derived *V* measures were consistently lower than those based on gut contents, especially in *C. albopunctata*. The differences

between the gut content and isotope-derived V measures could have arisen because not all individuals used in the isotope analyses were used in the gut content analyses (recall that some individuals had empty stomachs). Therefore, we double-checked our results by re-analyzing only the individuals for which we had both gut content and isotope data. We found no qualitative changes in the results (Table S2): the estimates of diet variation based on gut contents are overall slightly higher than those based on isotopes. Finally, the isotope model used is very sensitive to individuals showing signatures outside the range of prey signatures ('isotope outliers'; Araújo *et al.* 2007a). We removed the outliers and re-did the analyses. Overall, this procedure did not change substantially the results (Fig. S1).

MORPHOLOGY VS. DIET

We did not observe any significant correlations between body shape and diet overlap (Mantel; all P values > 0·408), indicating that the observed individual-level diet variation does not depend on the morphological characters examined here.

DIET VARIATION VS. POPULATION NICHE WIDTH

We found a positive relationship between TNW and V (slope ± SE = 0·236 ± 0·016; t = 15·089; P < 0·001; Fig. 3), indicating that populations with wider niches tend to be ecologically more variable. However, this relationship can be explained simply by stochastic sampling effects, as shown by a significant and even steeper null expectation
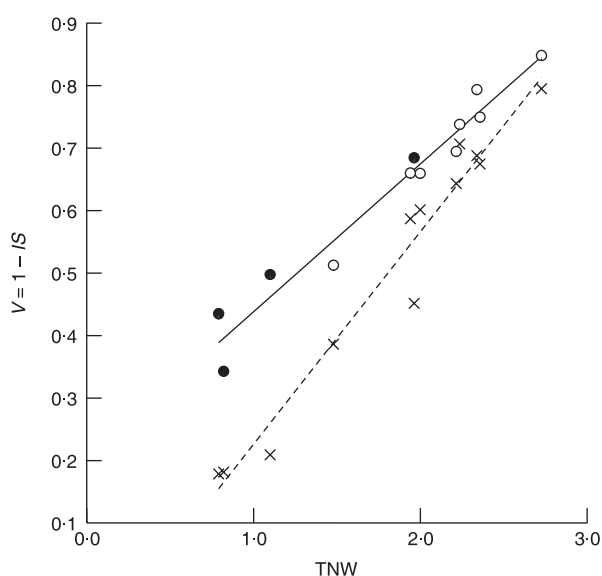
Fig. 3. Correlation between diet variation among individuals ($V = 1 – IS$) and Roughgarden's (1979) measure of population TNW. The empirical results are shown with circles. Filled circles: this study; empty circles: data from Bolnick *et al.* (2007). Crosses (and the dotted regression line) indicate the expected trend under a null model in which diet variation arises solely by individuals randomly sampling a limited set of prey from a shared prey distribution.

(slope ± SE = 0·340 ± 0·022; t = 15·748; P < 0·001; Fig. 3). It is worth mentioning that although the slopes of empirical and null relationships were similar, there was consistently higher diet variation in the observed than in the null samples (Fig. 3).

Discussion

PATTERNS OF RESOURCE USE

We did not observe any ontogenetic or sex-related diet differences, a pattern already observed in the four other frog species studied in the same community (Araújo *et al.* 2007b). Apparently, the morphological and/or behavioural differences between age classes and sexes are not important in determining the types of resources consumed in these frogs. Despite the lack of ontogenetic or sex-related diet differences, we found evidence of individual-level diet variation in all species. This increases the list of examples of individual specialization in frogs from four (Araújo *et al.* 2007b) to eight species, suggesting that this might be a common phenomenon in tropical frogs. This finding is in stark contrast to the current view that individual specialization is driven by ecological release in species-poor (e.g. islands, post-glacial lakes), generally temperate communities (Roughgarden 1974; Lister 1976; Ebenman & Nilsson 1982; Werner & Sherry 1987; Robinson *et al.* 1993; Smith & Skúlason 1996). Assuming that such examples of ecological release are very unlikely in the highly diverse, packed tropical communities, we should expect negligible individual specialization in tropical species. This expectation, however, is contrasted by the growing list of tropical species for which individual specialization has been reported. This list includes taxonomic groups as disparate as frogs (Araújo *et al.* 2007b; this study), a wasp (Araújo & Gonzaga 2007), and a marsupial (Martins *et al.* 2008). One possible explanation is that functional trade-offs might be a common feature of animal foraging and even stronger than previously thought, constraining individual niches to one or very few prey items. As a result, individual niches may correspond to subsets of the population niche even when the latter is not very large itself.

TEMPORAL CONSISTENCY

All studied species showed variation in $\delta^{13}C$ signatures spanning the range of $\delta^{13}C$ values of the consumed prey (Fig. 2), indicating long-term diet differences among individuals. These results were further confirmed by the isotope-derived measures of V (Table 2). Overall, isotopes indicated a smaller degree of diet variation than gut contents and in the case of *C. albopunctata*, the isotope model indicated little diet variation ($V_{exp} \sim 0·19$). One possible explanation for this discrepancy is the fact that gut contents may overestimate diet variation (Bolnick *et al.* 2002) if individuals can hold few prey items in stomachs. Isotopes in turn may underestimate diet variation if different resources have similar isotopic composition and cannot be discriminated by isotopes (Matthews & Mazumder 2004). In fact, some of the resources consumed by the frogs

have similar isotopic signatures (see Table S1 in Araújo *et al.* 2007a) and therefore cannot be discriminated with carbon isotopes. On the other hand, variation in assimilation, routing or fractionation among individuals (Gannes, O'Bryan & del Rio, 1997) may generate isotopic variation that is not related to diet. Unfortunately, we have no estimates of these sources of variation for our frogs. However, at least for fractionation, empirical data on different vertebrates show that among-individual variation tends to be low ($0.18‰^2$ in three-spined stickleback, Snowberg & Bolnick 2008; ~$0.4‰^2$ in birds, Hobson & Clark 1992; $0.54‰^2$ in the Barton Springs salamander, H. Gillespie, unpublished data). Other factors that may also increase isotopic variation include spatial and temporal changes in the isotopic baseline (Matthews & Mazumder 2005), which may account for part of the isotopic variation in the frogs. Finally, variation in isotopic composition within resources has been shown to affect the results of mixing models (Moore & Semmens 2008). Its impact on estimates of diet variation is unclear, but is likely to be low if individuals have consumed large numbers of prey items so that the variation within resources is averaged out in any given individual consumer. This tends to be the case when isotopes of consumers are estimated from tissues that integrate relatively long periods. Turnover rates in vertebrate muscle tissue are in the order of months (Dalerum & Angerbjörn 2005) and therefore it is unlikely that variation within resources had a major impact on our results. Bearing those limitations in mind, the method used here goes beyond a simple measure of isotopic variation in consumers (Fry *et al.* 1978; Layman *et al.* 2007) and is a useful complementary approach when coupled with other sources of information on diet (Araújo *et al.* 2007a; Woo *et al.* 2008). Regardless of the discrepancy between our gut-content and isotope data, both approaches indicate the presence of individual specialization in the studied populations.

MECHANISMS OF INDIVIDUAL–LEVEL DIET VARIATION

After detecting individual-level diet variation in a population, it is important to identify its underlying mechanisms. Diet variation among individuals stems from functional trade-offs that prevent any given individual from exploiting the whole set of available resources (Bolnick *et al.* 2003). These trade-offs are in general of three types: morphological, cognitive, or physiological (Schluter 1995; Estes *et al.* 2003; Olsson *et al.* 2007). We found no correlation between morphology and diet in the studied species, indicating that morphology is probably not the basis of diet variation in these species. We posit therefore that the mechanism of diet variation in these frogs is either cognitive or physiological. If individuals need to learn search images or how to handle prey and the amount of information an individual can handle is limited, learning trade-offs may prevent individuals from using multiple resources (Werner, Mittelbach & Hall 1981; Persson 1985; Lewis 1986; Werner & Sherry 1987; Bernays & Funk 1999). Moreover, ants are known to be highly toxic, hard-bodied prey (Caldwell 1996; Santos, Coloma & Cannatella 2003) that may be difficult to digest. Differences in toxin content and digestive requirements among prey may in turn form a basis for physiological trade-offs and generate individual-level diet preferences (Afik & Karasov 1995; Olsson *et al.* 2007). We acknowledge these suggestions are speculative at the moment, but both learning and physiological trade-offs can be tested experimentally. Only with quantitative information on costs of learning and/ or digestion of prey will we be able to determine the proximate mechanism of diet variation in these species.

DIET VARIATION VS. POPULATION NICHE WIDTH

Recently, Bolnick *et al.* (2007) described a general pattern in several animal taxa in which more generalist populations showed higher degrees of among-individual diet variation. This finding is relevant, because it suggests that more generalist populations, being more variable, may have more stable ecological dynamics (Lomnicki 1988; Kendall & Fox 2002, 2003; Fox 2005) and be subject to frequency-dependent interactions that may drive disruptive selection (Bolnick 2004; Pfennig *et al.* 2007; Bolnick & Lau 2008) and evolutionary divergence (Dieckmann & Doebeli 1999; Doebeli *et al.* 2007). Additionally, it gives support to a long-discredited hypothesis (NVH) according to which the expansion of population niches should be achieved via increased interindividual variation, so that 'much variation is probably adaptive in itself and is not part of the genetic or phenotypic load' (Van Valen 1965; p. 386). Bolnick *et al.* (2007) analysed four species of Brazilian frogs and found empirical evidence of a positive relationship between population niche width and diet variation that could not be accounted for by a null model. We redid their analyses, now adding four new species from the same localities, and could not reject the null hypothesis that this relationship is caused by stochastic sampling effects when there is limited information on individuals' diets. This is not to say that there is no such relationship in these frogs, but rather that with the amount of information we have, we cannot rule out our null hypothesis. Ideally, we should be able to conduct longitudinal sampling schemes, in which the same individuals are repeatedly sampled over long time periods (Werner & Sherry 1987; Estes *et al.* 2003; Tinker *et al.* 2007) to improve estimates of individual diets. It might be that these more thorough data sets allow the rejection of the null hypothesis, in support of the NVH. On the other hand, if we are still unable to reject the null hypothesis at least we will be more confident to assert that there is no such relationship between the population niche width and the degree of diet variation in these frogs. This would also indicate that functional trade-offs probably have different magnitudes in different species, precluding a direct association between population niche width and individual specialization across species.

We documented four new cases of significant diet variation in tropical frogs. These new examples are part of a growing list of species belonging to highly diverse communities showing degrees of diet variation comparable to those of depauperate, temperate communities (e.g. three-spined stickleback, $V \sim 0.6$; Svanbäck & Bolnick 2007). This pattern is at odds with

expectations from theory and suggest that functional trade-offs that constrain individual niches are more common and probably stronger than previously thought.

Acknowledgements

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Supporting information

Additional supporting information may be found in the online version of this article:

Fig. S1. Interpolation of *IS* from isotope variances: δ13C variances (Varδ13C) were estimated after the removal of isotope outliers and regressed onto measures of individual specialization (*IS*) of simulated populations (see text for details). Curves indicate fitted regressions; horizontal dashed lines indicate the empirical Varδ13C. Arrows indicate the empirical *IS* (emp) from gut contents and the expected *IS* (exp) interpolated from the empirical Varδ13C of four Brazilian frogs, using the regression equations. A *Physalaemus cuvieri* ($N = 49$); B *Eupemphix nattereri* ($N = 44$); C *Chiasmocleis albopunctata* ($N = 17$); D *Elachistocleis bicolor* ($N = 46$).

Table S1. Diet composition of *Chiasmocleis albopunctata* ($N = 50$), *Elachistocleis bicolor* ($N = 50$), *Eupemphix nattereri* ($N = 37$), and *Physalaemus cuvieri* ($N = 50$), in the Brazilian Cerrado, represented as the number of prey items consumed and their proportions (in parenthesis) in each species

Table S2. The *V* measure of individual specialization in the diets of four species of Brazilian frogs (*Physalaemus cuvieri*, *Eupemphix nattereri*, *Chiasmocleis albopunctata*, and *Elachistocleis bicolor*). Individual specialization was measured using both gut contents and δ13C stable isotopes. Only those individuals for which we had both gut content and isotope data were used. V_{emp}: empirical *V* index of individual specialization based on gut-content data; Varδ13C: empirically estimated variances of individual δ13C signatures; V_{exp}: expected value of the *V* index based on isotope data (see Methods). V_{emp} values were tested against null distributions generated with Monte Carlo bootstraps (10 000 simulations); ***$P < 0.001$. *n*: sample size

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