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VARIAÇÃO INTERINDIVIDUAL NO USO DE RECURSOS EM POPULAÇÕES NATURAIS: NOVOS PADRÕES E IMPLICAÇÕES

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Fortunate are those who have learned to see, in the wild things of nature, something to be loved, something to be wondered at, something to be reverenced, for they will have found the key to a never-failing source of recreation and refreshment.

—Hugh B. Cott

A hundred times every day I remind myself that my inner and outer life are based on the labors of other men, living and dead, and that I must exert myself in order to give in the same measure as I have received and am still receiving.

—Albert Einstein

RESUMO

A teoria ecológica clássica, em especial a teoria de nicho, foi construída sob a suposição de que os indivíduos de uma população são equivalentes em termos da utilização de recursos. Entretanto, é sabido que os indivíduos de uma população podem variar no uso de recursos e que essa variação pode ter importantes implicações ecológicas e evolutivas. Essa variação interindividual pode dar origem a morfotipos discretos ("polimorfismo de recursos") ou ser contínua ("especialização individual"). O presente estudo teve como objetivo investigar a variação interindividual no uso de recursos em quatro populações de rãs do Cerrado brasileiro (*Leptodactylus* sp., *L. fuscus*, *Eleutherodactylus* cf. *juipoca* e *Proceratophrys* sp.), uma população de vespas-caçadoras de uma área de Mata Atlântica (*Trypoxylon albonigrum*) e uma população do peixe lacustre *Gasterosteus aculeatus* da Columbia Britânica, Canada. Houve evidência de variação interindividual em todas as populações estudadas, indicando que esse fenômeno não é exclusivo de comunidades temperadas de baixa diversidade. Houve uma associação entre a amplitude dos nichos populacionais e o grau de variação interindividual, indicando que os nichos individuais permanecem estreitos apesar da expansão do nicho populacional. Esse padrão é consistente com a presença de *trade-offs* funcionais associados ao uso dos recursos. A base dos *trade-offs* permanece desconhecida no caso das rãs e das vespas, mas é provavelmente comportamental. No caso de *G. aculeatus*, os *trade-offs* têm base morfológica, mas são mediados pelo comportamento. Além disso, foi identificado um padrão de partição de recursos inédito nesses peixes, em que os indivíduos formam microguildas que representam subdivisões dos recursos litorâneos e pelágicos. São propostos dois novos métodos para a

investigação da variação intrapopulacional no uso de recursos, um deles baseado no uso de isótopos estáveis de carbono ($\delta^{13}C$) e o outro na teoria de redes complexas.

ABSTRACT

Ecological theory, and specially niche theory, was built on the assumption that individuals are equivalent in terms of resource use. However, the individuals in a population may vary in their resources, and this interindividual variation may have important ecological and evolutionary implications. Such variation may give rise to discrete morphological groups ("resource polymorphism") or it may be more continuous ("individual specialization"). In the present study, we investigated interindividual variation in resource use in four populations of frogs inhabiting the Brazilian Cerrado (*Leptodactylus* sp., *L. fuscus*, *Eleutherodactylus* cf. *juipoca* e *Proceratophrys* sp.), one population of hunting-wasp of the Atlantic Rainforest (*Trypoxylon albonigrum*), and one population of sticklebacks (*Gasterosteus aculeatus*) from British Columbia, Canada. We found evidence of interindividual diet variation in all studied populations, indicating that such variation is not restricted to temperate, depauperate comunities. There was an association between niche width and the degree of interindividual variation, indicating that individual niches remain constrained as the population niche expands. This pattern is consistent with the presence of functional trade-offs associated with resource use. In the case of the frogs and the wasps, the nature of the trade-offs remains unknown, but are likely to be behavioral. In the sticklebacks, the trade-offs have a morphological basis, but are mediated by behavior. We found that individual sticklebacks partition resources within littoral and within pelagic prey, which represents a finer pattern of resource partitioning than the traditional "littoral-pelagic" dichotomy. Two new methods for the

quantification of interindividual diet variation are proposed, one based on carbon stable isotopes ($\delta^{13}C$) and another based on complex-network theory.

SUMÁRIO

Introdução Geral

Teoria

A teoria ecológica clássica, em especial a teoria de nicho, foi construída sob a suposição de que os indivíduos de uma população são equivalentes em termos da utilização de recursos (Hutchinson 1957; Schoener 1989). De acordo com essa suposição, as distribuições de uso dos recursos dos indivíduos sobrepõem-se amplamente com a distribuição de uso de recursos da população. Essa suposição, que na física e na biologia matemática recebe o nome de "aproximação do campo médio" (Durrett & Levin 1994; Tilman & Kareiva 1997), traz a conveniência da simplicidade matemática e permite a modelagem de sistemas e fenômenos altamente complexos. Esse corpo teórico, no entanto, tem convivido com a percepção, há muito reconhecida na literatura ecológica, de que as populações naturais podem apresentar fenótipos que variam no uso dos recursos (Van Valen 1965; Schoener 1968; Roughgarden 1972, 1974).

Entre os fatores responsáveis por essa variação, o sexo e a idade têm sido apontados como os mais importantes, a ponto de Schoener (1986; p. 119) afirmar que "for the most part, the important between-phenotype variation in populations occurs between sex and age classes." Existe ainda um tipo de variação intrapopulacional no uso de recursos que pode se manifestar entre os indivíduos de mesmo sexo e idade. Por exemplo, muitas espécies de peixes de lagos temperados apresentam morfotipos discretos especializados em diferentes recursos e/ou habitats: existe um morfotipo litorâneo, associado a esse ambiente e especializado em macroinvertebrados bentônicos, e um morfotipo pelágico, que vive na coluna d'água e consome predominantemente zooplâncton (Skúlason & Smith 1995; Smith & Skúlason 1996; Skúlason *et al.* 1999; Robinson & Schluter 2000). Esse tipo de variação intrapopulacional foi chamado de "polimorfismo discreto no uso de recursos" ("discrete resource polymorphism"; Skúlason & Smith 1995; Smith & Skúlason 1996) e corresponde à existência de grupos discretos de indivíduos que podem ser identificados *a priori* com base em sua morfologia. Finalmente, existe um tipo de variação mais contínua, chamado de "especialização individual" ("individual specialization"; Bolnick *et al.* 2003), em que os indivíduos de uma população consomem apenas um subconjunto do espectro de recursos consumido pela população como um todo. Por exemplo, no tentilhão da ilha de Cocos, *Pinaroloxias inornata*, a população consome uma ampla variedade de recursos, mas cada indivíduo prefere e consome consistentemente apenas um ou dois tipos (Werner & Sherry 1987). Na verdade, o polimorfismo no uso de recursos e a especialização individual não são fundamentalmente diferentes, e, segundo Bolnick *et al.* (2003; p. 3), "In reality, individual variation and polymorphism are ends of a continuum of increasingly discrete variation."

A variação intrapopulacional no uso de recursos pode ter uma série de implicações ecológicas e evolutivas para as populações naturais (Bolnick *et al.* 2003). Por exemplo, ela pode reduzir a intensidade da competição intra-específica (Bolnick 2001; Swanson *et al.* 2003; Svanbäck & Bolnick 2007), já que a sobreposição de recursos entre os diferentes fenótipos é baixa. Além disso, se os indivíduos variam no uso de recursos, eles sofrerão efeitos diferenciados da competição interespecífica (Taper & Case 1985). Finalmente, alguns modelos de dinâmica populacional demonstraram que populações mais variáveis tendem a ser mais estáveis ao longo do tempo (Lomnicki 1988, 1992). Adicionalmente, a presença de variação intra-populacional pode gerar interações dependentes-de-freqüência (Dieckmann *et al.* 2004) responsáveis pela evolução de dimorfismo sexual (Bolnick & Doebeli 2003), morfotipos discretos (Smith & Skúlason 1996) e até mesmo divergência evolutiva (Dieckmann & Doebeli 1999; Bolnick 2006).

De acordo com alguns modelos de evolução de nicho, a presença de especialização individual na verdade não seria esperada na natureza (Roughgarden 1972, 1974; Taper & Case 1985), já que a expansão dos nichos populacionais deveria se dar via expansão dos nichos individuais e não via aumento da variação interindividual. Essa é uma expectativa intuitiva, já que os indivíduos generalistas seriam favorecidos em relação aos indivíduos especialistas por terem acesso a mais recursos e portanto maior capacidade de suporte. Se, no entanto, existem forças restringindo a expansão dos nichos individuais, espera-se observar especialização individual (Bolnick *et al.* 2003). Essa restrição à expansão dos nichos individuais pode se dar em função da presença de *trade-offs*, em que indivíduos que exploram de maneira eficiente um tipo de recurso são necessariamente ineficientes ao explorar outro tipo de recurso (Robinson *et al.* 1996). *Trade-offs* em geral têm base morfológica, resultando da interação entre as características biomecânicas dos indivíduos e os diferentes recursos (Price 1987; Smith 1990; Robinson *et al.* 1996). Por exemplo, em *Lepomis macrochirus*, um peixe de rios e lagos norte-americanos, os indivíduos maiores e com corpo mais alto (morfotipo litorâneo) são mais eficientes consumindo presas bentônicas (e.g. larvas de Chironomidae), ao passo que os indivíduos menores e mais delgados (morfotipo pelágico) são mais eficientes consumindo zooplâncton (Ehlinger 1990). Além disso, os *trade-offs* podem ter uma base comportamental, se o uso de recursos envolve aprendizado e os indivíduos têm limitações cognitivas (Werner *et al.* 1981; Werner & Sherry 1987), ou ainda uma base fisiológica, se os recursos mobilizam vias metabólicas específicas que envolvem ajustes fisiológicos individuais (West 1986, 1988; Afik & Karasov 1995). Esses *trade-offs*, por sua vez, impedem que os indivíduos usem todo o leque de recursos disponíveis, de forma que em populações fenotipicamente variáveis os indivíduos utilizarão apenas subconjuntos do nicho populacional, causando especialização individual (Bolnick *et al.* 2003).

Se esses *trade-offs* são um fenômeno geral, é razoável supor que populações com nichos mais amplos apresentem maior grau de especialização individual. Essa idéia corresponde à "hipótese da variação do nicho" ("niche variation hypothesis"; Van Valen 1965), segundo a qual populações que usam uma variedade maior de recursos seriam fenotipicamente mais variáveis, já que diferentes fenótipos seriam especializados em diferentes porções do nicho populacional (Van Valen 1965). Van Valen (1965) comparou populações continentais com populações ilhoas de seis espécies de aves e observou que as populações ilhoas, cujos nichos eram mais amplos do que o das populações continentais, possuíam bicos morfologicamente mais variáveis, com exceção de *Fringilla coelebs* nas Ilhas Canárias. Vale notar que a morfologia do bico determina o tipo de recurso alimentar consumido nesse sistema. Essa hipótese, apesar de ser intelectualmente atraente e de ter recebido algum suporte empírico (Van Valen 1965; Ebenman & Nilsson 1982; Werner & Sherry 1987), tem sido recorrentemente refutada em diferentes estudos envolvendo uma ampla gama de táxons (Grant 1979; Feinsinger & Swarm 1982; Patterson 1983; Dennison & Baker 1991; Diaz 1994; Simberloff *et al.* 2000; Blondel *et al.* 2002; Meiri *et al.* 2005). Contudo, uma falha dos testes da hipótese da variação do nicho conduzidos até o presente momento consiste na sua ênfase na morfologia como estimador da variação no uso de recursos. Na verdade, não há nenhuma razão lógica subjacente à hipótese de Van Valen (1965) que justifique o uso da variação morfológica como medida da variação no uso de recursos. A variação morfológica somente será uma boa estimativa da variação no uso dos recursos quando o uso de recursos se mapeia na morfologia, como no caso das aves estudadas por Van Valen (1965). Há casos, no entanto, em que a presença de variação interindividual no uso de recursos tem base exclusivamente comportamental, como no tentilhão da ilha de Cocos, *P. inornata*, em que não existe nenhuma associação entre a variação morfológica e a variação no uso de recursos (Werner & Sherry 1987).

De fato, existe na literatura a idéia de que a presença de especialização individual seria conseqüência da expansão dos nichos populacionais causada por liberação competitiva. Exemplos corroborando essa hipótese são comuns em peixes lacustres de regiões temperadas, como os esgana-gatas do gênero *Gasterosteus* (Schluter 1995; Robinson 2000; Bolnick 2004) e os Centrarchidae do gênero *Lepomis* (Robinson *et al.* 1993; Robinson *et al.* 1996). Nesses peixes, quando duas espécies vivem no mesmo lago, uma delas ocupa a região litorânea e se alimenta de presas do bentos, enquanto que a outra ocupa a zona pelágica e se alimenta de zooplâncton (Robinson *et al.* 1993). Se uma das espécies está ausente, no entanto, a espécie presente explora os dois microhabitats, o que tem sido interpretado como evidência indireta de expansão do nicho populacional causada por liberação competitiva. A expansão do nicho populacional, por sua vez, se daria via aumento da variação interindividual, em vez da expansão dos nichos individuais (Ebenman & Nilsson 1982; Werner & Sherry 1987; Robinson *et al.* 1993) levando ao surgimento de especialização individual. Esses exemplos levaram à idéia generalizada de que o fenômeno da especialização individual decorre da invasão de nichos "vagos"

(Smith & Skúlason 1996) geralmente em comunidades temperadas depauperadas (Ebenman & Nilsson 1982; Robinson *et al.* 1993; Schluter 1995; Robinson *et al.* 1996; Robinson 2000). Os dois únicos exemplos tropicais, o tentilhão da ilha de Cocos *P. inornata* (Werner & Sherry 1987) e o tentilhão africano *Pyrenestes ostrinus* (Smith 1990), fazem parte de comunidades altamente depauperadas. De acordo com a idéia vigente, portanto, não seria esperado esse tipo de variação em comunidades diversas e saturadas de espécies potencialmente competidoras, como as comunidades tropicais.

Recentemente, Bolnick *et al.* (2003) demonstraram a presença de variação interindividual em um grande número de táxons de vertebrados e invertebrados. Esses autores chamam a atenção para o fato de que esses estudos, apesar de demonstrarem a existência desse tipo de variação, rejeitando a hipótese nula de que os indivíduos da população são equivalentes no uso de recursos, não trazem nenhuma medida do *grau* de variação interindividual nessas popoulações. A quantificação desse tipo de variação, por sua vez, poderia contribuir para uma melhor compreensão dos fatores ecológicos (e.g. número de espécies competidoras/predadoras na comunidade, presença de dimorfismo sexual) responsáveis pelo surgimento e manutenção dessa variação em populações naturais. Alguns índices para medir esse tipo de variação foram propostos recentemente (Bolnick *et al.* 2002) e seu uso pode ajudar a responder a algumas dessas importantes questões. Esses índices, no entanto, não permitem discernir os casos em que a variação no uso de recursos é discreta (polimorfismo discreto) dos casos em que ela é contínua (especialização individual), se a variação morfológica da população é contínua e unimodal (ausência de morfotipos discretos). Por exemplo, entre as espécies de peixes lacustres temperados que têm variação intrapopulacional no uso de recursos, há espécies que de fato apresentam morfotipos discretos (Skúlason & Smith 1995; Smith & Skúlason 1996; Skúlason *et al.* 1999; Robinson & Schluter 2000) que podem ser facilmente identificados *a priori*. Há outras, no entanto, em que a variação morfológica é contínua, e os morfotipos (e.g. litorâneo e pelágico) na verdade correspondem aos extremos de uma distribuição unimodal (Robinson 2000; Svanbäck & Bolnick 2007). Nestas últimas, não se sabe, na verdade, se a variação no uso dos recursos também é contínua, sendo um mero reflexo da variação morfológica, ou se ela é discreta, refletindo a discretização do ambiente, mas passa despercebida por não se mapear diretamente na morfologia. Seria útil, portanto, dispor de um método que nos permitisse quantificar o grau de discretização no uso dos recursos que não dependesse da identificação *a priori* de morfotipos.

Consistência Temporal

Uma importante suposição que se faz em estudos de variação intrapopulacional no uso de recursos é a de que as estimativas dos nichos individuais são representativas do uso de recursos pelo indivíduo a longo prazo (Bryan & Larkin 1972; Roughgarden 1974; Robinson *et al.* 1993; Schindler 1997; Fermon & Cibert 1998). Idealmente, para se quantificar esse tipo de variação na natureza, os indivíduos deveriam ser amostrados repetidamente (amostras longitudinais), o que em muitos casos não é possível ou no mínimo extremamente difícil e custoso. Na maioria dos casos, os indivíduos são amostrados uma única vez (amostras pontuais), e supõe-se que essa "fotografia" dos nichos individuais é representativa do uso de recursos por parte dos indivíduos no longo prazo. Esse é o caso, por exemplo, dos estudos baseados em conteúdos estomacais, uma forma relativamente fácil e direta de estudar dietas e consagrada na literatura ecológica. Há exemplos da literatura demonstrando que conteúdos estomacais podem ser bons estimadores do uso de recursos no longo prazo (e.g. Bryan & Larkin 1972; Schindler 1997). Há casos, no entanto, em que os conteúdos estomacais tendem a subestimar os nichos individuais, fazendo com que os indivíduos aparentem ser mais especialistas do que de fato são ("falsos especialistas"; Warburton *et al.* 1998). Por exemplo, Warburton *et al.* (1998) analisaram conteúdos estomacais da perca-prateada *Bidyanus bidyanus* e observaram que os indivíduos eram altamente especialistas, mas apenas durante períodos de 2-4 semanas, quando mudavam suas preferências alimentares em resposta a flutuações nas abundâncias dos recursos. Em casos como esse, amostras pontuais farão com que os indivíduos aparentem ser mais especialistas do que de fato são, levando a uma superestimativa do grau de especialização individual (Bolnick *et al.* 2002; Bolnick *et al.* 2003).

Em estudos sobre variação intrapopulacional baseados em amostras pontuais, portanto, é importante aplicar métodos para inferir a consistência temporal no uso dos recursos (Bolnick *et al.* 2003). Uma das maneiras de inferir a consistência temporal no uso dos recursos é por meio de correlações entre dieta e morfologia (Robinson *et al.* 1993; Fermon & Cibert 1998; Svanbäck & Bolnick 2007). Essas correlações indicam que o tipo de recurso consumido pode ser previsto pela morfologia dos indivíduos, e, conseqüentemente, a variação interindividual observada não é um simples artefato de amostragem (Bolnick *et al.* 2003). Uma outra abordagem utilizada é a quantificação de isótopos estáveis nos consumidores e em seus recursos (Fry *et al.* 1978; Gu *et al.* 1997). A utilidade dos isótopos estáveis reside no fato de que é possível discriminar os recursos consumidos por um predador com base na sua composição isotópica. Como os isótopos têm uma taxa de decaimento relativamente lenta nos tecidos animais, na escala de meses no caso de tecido muscular e podendo chegar a vários anos no caso de osso e cartilagem (Tieszen *et al.* 1983; Dalerum & Angerbjörn 2005), eles não estão sujeitos aos efeitos estocásticos associados aos conteúdos estomacais.

Alguns estudos usaram a variância das assinaturas isotópicas como medida da variação interindividual na dieta (Fry *et al.* 1978). A variância isotópica da população, no entanto, não depende apenas da variação na dieta dos indivíduos, mas também da variação da composição isotópica dos recursos (Matthews & Mazumder 2004). Isso significa que populações apresentando o mesmo grau de variação interindividual na dieta, mas cujos recursos apresentem diferentes graus de variação isotópica, apresentarão diferentes valores de variância isotópica. Em outras palavras, existe um componente da variância isotópica das populações que não necessariamente está relacionado a variação da dieta. As variâncias isotópicas, portanto, não podem ser usadas diretamente como medida de variação interindividual na dieta e muito menos comparadas em seus valores de face entre diferentes populações. Seria útil, portanto, um método que permitisse transformar as variâncias isotópicas em medidas comparáveis entre populações, como forma de entender que condições ecológicas determinam a existência desse tipo de variação em populações naturais.

Objetivos

O presente estudo teve como objetivo geral investigar a existência de variação interindividual no uso de recursos em diferentes populações naturais, bem como quantificar o grau dessa variação. Especificamente, foi testada a hipótese de que populações tropicais em comunidades altamente diversas não apresentam variação interindividual no uso de recursos, já que a competição interespecífica restringiria a expansão dos nichos populacionais, mantendo-os estreitos, e, dessa forma, impediria o surgimento de variação interindividual. Para testar essa hipótese, estudou-se a variação interindividual na dieta de quatro espécies de rãs (Figura 1) que compõem comunidades de anuros altamente diversas em duas áreas de Cerrado no município de Uberlândia, Minas Gerais, nomeadamente, *Leptodactylus* sp., *L. fuscus*, *Eleutherodactylus* cf. *juipoca* e *Proceratophrys* sp. (Capítulo 1). Os anfíbios anuros têm sido modelos clássicos em estudos de dieta e ecologia alimentar (Toft 1980a, 1980b, 1981, 1985). Além disso, formam um grupo taxonômico altamente diversificado na região tropical (Duellman & Trueb 1986), e, nesse sentido, constituem um modelo adequado para se testar a referida hipótese. Havia, no entanto, uma questão amostral importante a ser considerada: os exemplares das rãs estudadas foram sacrificados para a obtenção de seus conteúdos estomacais. Esses conteúdos estomacais, por sua vez, constituem amostras pontuais do uso de recursos dos indivíduos, não havendo nenhuma garantia de que eles de fato representam as preferências individuais de longo prazo. Era necessário, portanto, respaldar os resultados obtidos com uma abordagem complementar, que permitisse inferir a consistência temporal no uso de recursos dos indivíduos. O Capítulo 2 apresenta um novo método para a quantificação da variação interindividual na dieta baseado no uso de isótopos estáveis de carbono ($\delta^{13}C$). Os isótopos estáveis apresentam características que os tornam uma ferramenta poderosa para a determinação das dietas individuais em escalas de tempo muito superiores àquela revelada pelos conteúdos estomacais. Para que esse método possa ser usado por pesquisadores em geral, o programa de computador VarIso1 foi implementado em linguagem C, e seu manual encontra-se no Apêndice 1. Esse método foi aplicado aos dados de conteúdos estomacais analisados no Capítulo 1, corroborando seus resultados. Com o objetivo de estabelecer o grau de generalidade desse fenômeno em comunidades tropicais, um outro sistema foi estudado, desta vez um invertebrado da Mata Atlântica, a vespa-caçadora *Trypoxylon albonigrum* (Figura 1). Essa vespa constrói ninhos de barro que são provisionados com um grande número de aranhas. Esses ninhos constituem um registro natural das preferências individuais nessa espécie, fazendo desse sistema um excelente modelo para o estudo da variação interindividual no uso de recursos. Os resultados referentes a esse estudo são apresentados no Capítulo 3, que documenta mais um caso de variação interindividual no uso de recursos em comunidades tropicais de alta diversidade e o primeiro caso em uma espécie de vespa-caçadora. O Capítulo 4 dedica-se à investigação de uma questão até então não resolvida sobre o polimorfismo no uso de recursos em peixes de lagos pós-glaciais do hemisfério norte. Foi usado como modelo de estudo uma população do esgana-gata *Gasterosteus aculeatus* (Figura 1) do lago Blackwater, Columbia Britânica, Canada. Foi desenvolvida uma nova abordagem, baseada na teoria de redes complexas, que permite a detecção e quantificação de polimorfismos discretos no uso de recursos mesmo quando a variação morfológica da população é unimodal. O manual do programa DIETA1, desenvolvido com o objetivo de aplicar esse método, está disponível no Apêndice 2.

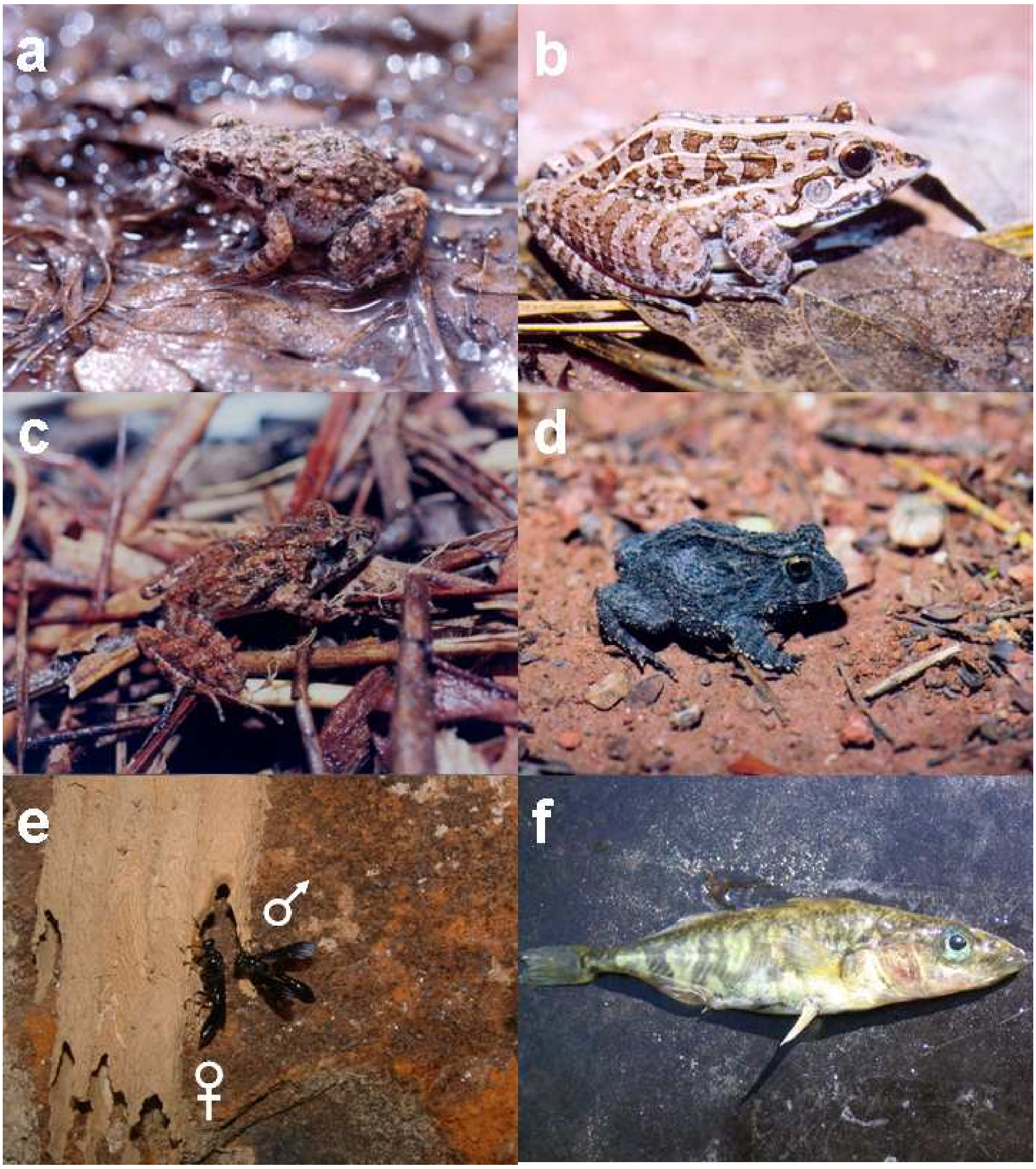

Figura 1. Espécies analisadas no presente estudo: **a** *Leptodactylus* sp.; **b** *Leptodactylus fuscus*; **c** *Eleutherodactylus* cf. *juipoca*; **d** *Proceratophrys* sp. (Fotos: Ariovaldo A. Giaretta); **e** casal de *Trypoxylon albonigrum* na entrada do ninho de barro em forma de tubo (Foto: Marcelo O. Gonzaga); **f** *Gasterosteus aculeatus* da Columbia Britânica, Canada (Foto: Daniel I. Bolnick). Fotos fora de escala.

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Capítulo 1

Intrapopulation diet variation in four frogs (Leptodactylidae) of the Brazilian savannah
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Copeia (no prelo)

Intrapopulation Diet Variation in Four Frogs (Leptodactylidae) of the Brazilian Savannah

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ABSTRACT

Age and sex-based as well as individual-level diet variation are known to occur in many natural populations, and may have important ecological and evolutionary implications. In the case of individual-level diet variation, most examples come from species-poor, temperate communities, and it is currently believed that it results from population niche expansion following interspecific competitive release. We investigated and measured the intrapopulation diet variation in four species of frogs, *Leptodactylus* (= *Adenomera*) sp., *Eleutherodactylus* cf. *juipoca*, *L. fuscus*, and *Proceratophrys* sp., that are part of species-rich frog communities of the Brazilian Cerrado. Specifically, we investigated age and sex-related, as well as individual-level diet variation. We measured individual-level diet variation with the *IS* index of individual specialization, which is a measure of the degree of overlap between individual niches and the population niches. We found no ontogenetic shifts or sex-related differences in the types of prey consumed. However, we found evidence of individual-level diet variation in the four studied species (*IS* ~ 0.2 - 0.5).

There was a negative correlation between *IS* and the population niche width ($r = -0.980$; $P < 0.0001$), indicating that interindividual diet variation is more pronounced in more generalized populations. This pattern suggests that individual niche widths remain constrained even when population niche breadth is wide, consistent with the presence of functional trade-offs. We found no evidence that these trade-offs arise from morphology, since there was no diet-morphology correlation. We hypothesize that trade-offs have a behavioral or physiological basis, which needs further investigation. This is the first documented case of individual-level diet variation in a diverse tropical community, indicating that this phenomenon is not restricted to competitive release-driven niche expansion in temperate, depauperate communities.

Key-words: anurans; *Cerrado*; individual specialization; resource partitioning; seasonal variation

RESUMO

A variação entre classes etárias, entre os sexos ou mesmo entre os indivíduos de uma população é um fenômeno comum na natureza, tendo implicações ecológicas e evolutivas importantes. A maioria dos exemplos de variação interindividual conhecidos ocorre em comunidades temperadas pouco diversas, e acredita-se que esse tipo de variação resulta da expansão do nicho populacional gerada por liberação competitiva. Nós medimos o grau de variação intra-populacional na dieta de quatro espécies de rãs, *Leptodactylus* (= *Adenomera*) sp., *Eleutherodactylus* cf. *juipoca*, *L. fuscus* e *Proceratophrys* sp., pertencentes a comunidades de anuros de alta diversidade do Cerrado brasileiro. Nós medimos o grau de variação interindividual com o índice *IS* de especialização individual, que mede o grau de sobreposição entre os nichos individuais e o nicho populacional. Não houve mudança ontogenética nem diferença entre os sexos quanto ao tipo de recurso consumido. Houve, no entanto, variação interindividual na dieta das quatro espécies estudadas (*IS* ~ 0.2 - 0.5). Houve correlação negativa entre *IS* e a amplitude do nicho populacional, indicando que o grau de especialização individual é maior nas populações mais generalistas. Isso indica que os nichos individuais permanecem estreitos mesmo que o nicho populacional seja amplo, o que sugere a presença de *trade-offs*. De modo geral, não houve correlação entre a dieta e a morfologia dos indivíduos, indicando que os *trade-offs* não têm base morfológica. Nós hipotetizamos que os *trade-offs* têm base comportamental e/ou fisiológica, o que precisa ser investigado. Esse é o primeiro relato de variação interindividual em comunidades tropicais de alta diversidade, indicando que esse fenômeno não necessariamente resulta da expansão do nicho populacional resultante de liberação ecológica, em regiões temperadas pouco diversas.

INTRODUCTION

Variation in resource use between age classes and sexes has been widely documented in the literature (e.g., Schoener 1968, 1986). Age-based niche variation may be a result of changes related to size or development (Lima 1998; Lima & Magnusson 1998), whereas sex-based variation in resource use may result from differences in morphology (Shine *et al.* 2002), or differences in behavior or energetic requirements related to reproduction (Belovsky 1978; Martins *et al.* 2006). However, there is also a type of variation in resource use that cannot be attributed to sex or age and has been termed “individual specialization” (Bolnick *et al.* 2003). For example, individuals of the Cocos Finch, *Pinaroloxias inornata*, consistently use only a subset of the resources used by the population as a whole (Werner & Sherry 1987). This phenomenon may have several ecological and evolutionary implications (Bolnick *et al.* 2003), such as the relief of intraspecific competition (Smith 1990; Swanson *et al.* 2003) or the generation of frequency-dependent interactions that may drive populations to divergence (Dieckmann & Doebeli 1999; Bolnick & Doebeli 2003a; Svanbäck & Bolnick 2005).

According to theory, we would not expect to find individual specialization in natural populations (Roughgarden 1972, 1974; Taper & Case 1985), unless there are constraints on individuals' niche widths (Bolnick *et al.* 2003). Constraints generally arise from functional trade-offs, in which consumers efficiently exploiting one type of resource are inefficient using another type of resource (Robinson *et al.* 1996). Trade-offs are generally associated to the functional morphology of consumers (Price 1987; Smith 1990; Robinson *et al.* 1996), but may also have a behavioral or physiological basis (Bolnick *et al.* 2003). For example, in the Bluegill Sunfish *Lepomis macrochirus*, individuals with deeper bodies are more efficient in feeding on benthic prey, whereas more streamlined individuals perform better on zooplankton (Ehlinger 1990). Such trade-offs prevent individuals from using the full range of available resources, so that in phenotypically variable populations individuals may use different subsets, resulting in individual specialization. In this case, we would expect populations with broader niches to show higher degrees of individual specialization, which has been observed in *Anolis* lizards (Lister 1976; Roughgarden 1979). It would be informative to know how general this pattern is in natural populations.

The evolution of individual specialization has been interpreted as a consequence of the population niche expansion following interspecific competitive release. Examples supporting this idea are common in temperate lacustrine fish, such as sticklebacks

(Schluter 1995; Robinson 2000; Bolnick 2004) and sunfish (Robinson *et al.* 1993; Robinson *et al.* 1996). In these fishes, when two species occur in the same lake, one of them occupies the littoral microhabitat and feeds on benthic prey, whereas the other is limnetic and feeds on zooplankton (Robinson *et al.* 1993). If one of the species is absent, however, the present species exploits both benthic and limnetic niches, indicating competitive release-driven niche expansion. This niche expansion is often achieved by increased between-individual variation, rather than increased niche width of all individuals (Ebenman & Nilsson 1982; Robinson *et al.* 1993). Therefore, it is currently believed that the occurrence of individual specialization is a consequence of population niche expansion following the invasion of "empty" niches (Smith & Skúlason 1996), generally in species-poor, temperate communities (Ebenman & Nilsson 1982; Robinson *et al.* 1993; Schluter 1995; Robinson *et al.* 1996; Robinson 2000). The few tropical taxa known to exhibit diet variation are also in species-poor communities (Roughgarden 1974; Werner & Sherry 1987).

To evaluate whether individual specialization really is stronger in low-diversity environments, one must be able to compare the degree of diet variation across systems. This comparison requires that, instead of simply testing for the presence of individual specialization, ecologists should actually try to measure it in a standardized manner (Bolnick *et al.* 2003). Bolnick et al. (2002) proposed indices to quantify individual-level diet variation and stirred researchers to use them when investigating individual specialization. In the present study, we investigated the intrapopulation diet variation in four species of Neotropical frogs inhabiting species-rich (around 20 frog species; Giaretta et al., unpubl.) communities of the highly seasonal Brazilian Cerrado (Oliveira &

Marquis 2002). The character-release hypothesis suggests that the studied species should show negligible degrees of individual specialization. Specifically, we (1) tested for the presence of ontogenetic diet shifts; (2) tested for the presence and measured the degree of sex-related and individual-level diet variation; (3) tested the hypothesis that higher degrees of individual specialization are associated with broader niches; (4) tested if morphology is the underlying mechanism of individual specialization; and finally (5) tested the effect of season on the frogs' diets.

MATERIALS AND METHODS

Study system

We analyzed the stomach contents and morphology of four species of frogs from a tropical savannah formation in southeastern Brazil (Cerrado; Oliveira & Marquis 2002). A wet/warm season (henceforth "wet season") from September to March and a dry/mild season (henceforth "dry season") from April to August characterize the local climate. The mean annual precipitation is 1550 mm (range 750 - 2000), and in the dryer months can be zero (Rosa *et al.* 1991). The monthly mean temperature ranges from 19 to 30C, and winter frosts are frequent (Giaretta & Menin 2004).

Specimens from four species, namely, *Leptodactylus* (= *Adenomera*) sp. (*n* = 104 individuals), *Eleutherodactylus* cf. *juipoca* (*n* = 115), *L. fuscus* (*n* = 86), and *Proceratoprhys* sp., (*n* = 55), were collected in the municipality of Uberlândia (18º 55'S, 48º 17'W; 850 m), in the state of Minas Gerais, southeastern Brazil. The collection sites were located at the Clube de Caça e Pesca Itororó de Uberlândia, CCP (*Leptodactylus* sp., *L. fuscus*, and *Proceratophrys* sp.), and at the Estação Ecológica do Panga, EEP (*E.* cf. *juipoca*), two of the few remnants of original savannah vegetation still present in the municipality (Goodland & Ferri 1979), characterized by shrubby grassland areas surrounding wet areas such as *veredas* (marshes with buriti-palms *Mauritia flexuosa*) or temporal and permanent ponds (França *et al.* 2004; Giaretta & Menin 2004; Kokubum & Giaretta 2005). Frogs were collected weekly in the wet season and once every two weeks in the dry season, for a period of two years; specimens of *Leptodactylus* sp. were collected from October 1999 to March 2001; *E.* cf. *juipoca* from September 2000 to October 2001; *L. fuscus* from October 1999 to December 2000; and *Proceratophrys* sp. from November 1999 to August 2001. Frogs were captured with pitfall traps containing 5% formalin in order to prevent the digestion of gut contents and later transferred to 70% ethanol. Voucher specimens were deposited at the collection of the Museu de Biodiversidade do Cerrado of the Universidade Federal de Uberlândia (AAG-UFU).

Data collection

Frogs were dissected to obtain stomach contents. Prior to dissection, five measurements were taken from each specimen with a digital caliper (nearest 0.01 mm) always by the same person (M.S. Araújo): snout-vent length (SVL), mouth width (MW), lower jaw length (LJ), head length (HL) and eye-nostril distance (EN). We did not measure the mass of individuals, because preservative absorption was likely to bias our results. Diets were quantified by the analysis of stomach contents of the preserved specimens. Prey items were counted, and identified to the lowest taxonomic level possible (family level in most cases). Upon dissection, individuals were sexed by examination of gonads. In a few cases we failed to determine the sex of individuals, which explains the differences in sample sizes between Tables 1 and 2.

Data analyses

We found 97 prey taxa, spanning many families and orders, mostly insects and arachnids. Within orders, we grouped several families into functional groups, based on microhabitat (e.g., aquatic, soil, vegetation) and morphotypes (e.g., alates or workers of Isoptera), and ended up with 46 prey categories (Appendix 1). Within each frog species, most categories had very little abundances in the diets, so we used a rule of thumb (Krebs 1989) in order to eliminate those poorly represented categories, retaining the most representative ones. This rule consists of calculating the reciprocal of the number k of prey categories consumed ($1/k$), and using it as a cut-off value for the inclusion of prey categories in further analyses. A category j is included if its proportion in the population diet $q_j \geq 1/k$. The cut-off value was 0.03 in all frog species, so that only categories representing less than 3% of diet items in each species were eliminated.

In order to investigate ontogenetic diet shifts, we took an approach that relates body size (a proxy for age) to interindividual diet overlap. Within each species, we first did a Principal Components Analysis (PCA) on the five log-transformed morphological measurements. We then took the PC1 scores (interpreted as size) and calculated the Euclidean distances between all pairs of individuals, which generated a matrix of individual pairwise size distances. Next, we calculated a measure of pairwise diet overlap among individuals based on Schoener's (1968) proportional similarity index (PS),

$$PS_{ij} = 1 - 0.5\sum_{k} \left| p_{ik} - p_{jk} \right| ,$$

in which p_{ik} and p_{jk} are the proportions of prey category k in individual i's and j's diet, respectively. PS_{ij} is the proportional similarity between the diets of individuals i and j, varying from 0 (no overlap) to 1 (total overlap). If there are diet shifts associated with body size, which would indicate ontogenetic diet shifts, we would expect that more similar sized individuals have more similar diets. If this is true, we would expect the matrix of body size differences to be negatively correlated with the matrix of pairwise diet overlap, since the higher the size distance the lower the diet overlap. We tested for the correlation between matrices with a simple Mantel test with 1,000 simulations.

We tested for sex-related differences in diet also using the *PS* index, in which p_{ik} and p_{jk} represent the proportion of prey category k in males' and females' diets, respectively. In order to detect possible seasonal changes in the degree of sex-related diet variation, we analyzed samples from the wet and dry seasons separately. In the dry season, small sample sizes prevented us from analyzing *E.* cf. *juipoca* ($n = 2$ males), and *Proceratophrys* sp. ($n = 3$ females; $n = 5$ males).

When measuring individual-level diet variation, we also kept samples separated by season. We did this because the degree of individual-level diet variation may be affected by variations in resource abundance (Svanbäck & Persson 2004; Svanbäck & Bolnick 2005), and we know that the abundance of terrestrial arthropods varies seasonally in the Cerrado (Pinheiro *et al.* 2002; G. Machado, unpubl.). We used the proposed adaptation of *PS* to measure individual-level diet variation (PS_i), which measures the overlap between an individual i's diet and the population diet (Bolnick *et al.* 2002). In the case of PS_i, p_{ik} represents the proportion of category k in individual i's diet and p_{jk} is replaced by q_k, the proportion of category k in the population diet. For an individual i that specializes on a single prey category k, its PS_i will take on the value of the proportion of prey k in the population, whereas for individuals that consume prey in direct proportion to the population as a whole PS_i will equal 1. 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). An interesting feature of PS_i is that it generates measures of individual specialization for each individual in the population, which allowed us to compare the degree of individual specialization between wet and dry seasons by performing a Mann-Whitney *U* test on PS_i values.

In order to test the hypothesis that the degree of individual specialization increases with the population niche width, we did a Pearson's correlation test between the calculated *IS* measures and Roughgarden's (1979) index of total niche width (*TNW*), which uses the Shannon-Weaver diversity index as a measure of the population variance in resource use. If the degree of individual specialization increases with the expansion of the population niche, we would expect a negative correlation between *IS* and *TNW* (recall that lower *IS* values indicate higher individual specialization).

In order to test the hypothesis that individual-level diet variation is based on morphological trade-offs, we correlated the previously calculated matrix of pairwise diet overlaps with a matrix of Euclidean morphological distances based on all but PC1 scores (interpreted as body shape). If there is an effect of functional morphology on diet, we would expect that morphologically similar individuals (small distances) also show similar diets (high diet overlap), and vice-versa. If this is 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 1,000 simulations. Finally, we did a MANOVA on the arcsine square-root transformed proportions of prey categories in individual diets, with prey categories as the dependent variables and season as the independent variable to test for seasonal variation in the frogs' diets.

The calculation of all indices was performed in IndSpec1, a program to calculate indices of individual specialization (Bolnick *et al.* 2002). We also used IndSpec1 to calculate the significance of the *PS* measures between sexes and the *IS* measures of individual specialization. IndSpec1 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 1,000 replicates in Monte Carlo bootstrap simulations to obtain *P*-values for these indices. The PCA, the Mann-Whitney *U* test, the Pearson's correlation analysis, and the MANOVA were performed in SYSTAT11; the Mantel tests were carried out using the software PopTools 2.6.9 (Hood, G. M. 2005. PopTools version 2.6.9. Available on the internet. http://www.cse.csiro.au/poptools).

RESULTS

The correlation between body size and diet overlap was not significant in any of the analyzed species (Mantel; all *P*-values > 0.104), indicating that there are no ontogenetic shifts in the prey categories consumed. None of the *PS* measures between sexes differed significantly from the Monte Carlo null expectations (all *P*-values > 0.482; Table 1), indicating the absence of sex-related differences in diet in the analyzed species. However, there was evidence of significant individual specialization in the three analyzed species, except in *Proceratophrys* sp. in the dry season (Table 2). Individual specialization was significantly weaker (higher *IS*) in the dry season in *Leptodactylus* sp. (Mann-Whitney $U = 900$; $P = 0.003$; Fig. 1A) and *Proceratophrys* sp. ($U = 287$; $P = 0.002$; Fig. 1D). *Eleutherodactylus* cf. *juipoca* showed the same trend, but the difference was only marginally significant ($U = 1743$; $P = 0.052$; Fig. 1B), whereas *L. fuscus* showed an opposite trend, but not significant ($U = 525$; $P = 0.263$; Fig. 1C). There was a significant negative correlation between *IS* and *TNW* (Pearson's $r = -0.980$; $P < 0.0001$; $n = 8$), indicating that the wider the population niche the higher the degree of individual specialization. We did not observe any significant correlations between body shape and diet overlap (Mantel; all P-values > 0.194), indicating that the observed individual-level diet variation has no morphological basis. There was no effect of season on the diets of *Leptodactylus* sp. (MANOVA; Wilk's $\Lambda = 0.895$; $P = 0.319$), *L. fuscus* (Wilk's $\Lambda = 0.889$; $P = 0.791$), and *Proceratophrys* sp. (Wilk's $\Lambda = 0.706$; $P = 0.697$), but a marginally significant effect in *E.* cf. *juipoca* (Wilk's $\Lambda = 0.818$; $P = 0.062$).

Table 1. Schoener's (1968) proportional similarity index (*PS*) between the diets of males and females of four species of Brazilian frogs [*Leptodactylus* (= *Adenomera*) sp., *Eleutherodactylus* cf. *juipoca*, *L. fuscus*, and *Proceratophrys* sp.]. Comparisons were made in the wet and dry seasons. *P*-values were obtained in Monte Carlo bootstraps (1,000 simulations). *n*: number of frog specimens.

Species	Wet season			**Dry season**		
	PS	*P*	*n*	*PS*	*P*	*n*
Leptodactylus sp.	0.8251	0.823	86	0.4437	0.482	14
E. cf. *juipoca*	0.7402	0.779	54	–	–	–
L. fuscus	0.7157	0.826	62	0.4886	0.818	20
Proceratophrys sp.	0.6293	0.958	42	–	–	–

Table 2. *IS* measure of individual specialization in the diet of four species of Brazilian frogs [*Leptodactylus* (= *Adenomera*) sp., *Eleutherodactylus* cf. *juipoca*, *L. fuscus*, and *Proceratophrys* sp.]. Individual specialization was measured in the wet and dry seasons. *P*-values were obtained in Monte Carlo bootstraps (1,000 simulations). *n*: number of frog specimens.

Species	Wet season			**Dry season**		
	IS	*P*	*n*	*IS*	*P*	*n*
Leptodactylus sp.	0.3407	< 0.001	86	0.4875	< 0.001	14
E. cf. *juipoca*	0.2621	0.016	54	0.3054	0.011	53
L. fuscus	0.2507	< 0.01	63	0.2065	< 0.001	20
Proceratophrys sp.	0.1521	< 0.001	42	0.3401	0.10	8

DISCUSSION

We found no age or sex-related diet differences in any of the four studied species. However, we found evidence of significant individual specialization in all species. Additionally, the degree of individual specialization seems to vary among species and seasons. The morphological traits we investigated were not good predictors of individuals' diets, suggesting that among-individual diet variation may not have a morphological basis. In the following paragraphs we discuss (i) the observed patterns of intrapopulation diet variation in the studied frogs; (ii) the possible mechanisms underlying the observed diet variation; and (iii) the temporal consistency of individual-level diet variation.

Patterns of intrapopulation diet variation

Ontogenetic shifts in prey type, which have been reported for several Amazonian frogs and may have important implications in terms of resource partitioning at both the population and community level (Lima & Moreira 1993; Lima 1998; Lima & Magnusson 1998; Biavati *et al.* 2004) were not observed in our study. Apparently, the differences in body size as well as possible differences in behavior between juveniles and adults are not important in determining the types of food consumed in the studied species. Likewise, sex seems not to be an important factor in food consumption in the studied frogs, which was also observed in other frog species (Lima & Moreira 1993; Biavati *et al.* 2004) and perhaps is a general trend in frogs. In spite of the overall lack of age and sex-related diet differences, we did find evidence of individual-level variation in all species. In the only exception, *Proceratophrys* sp. in the dry season, we probably lacked statistical power due to the very small sample size ($n = 8$; Table 2). Our results, therefore, suggest that frog populations, although exploiting a variety of food taxa (many arthropod orders and families), may be actually composed of individuals with different preferences or prey capture abilities.

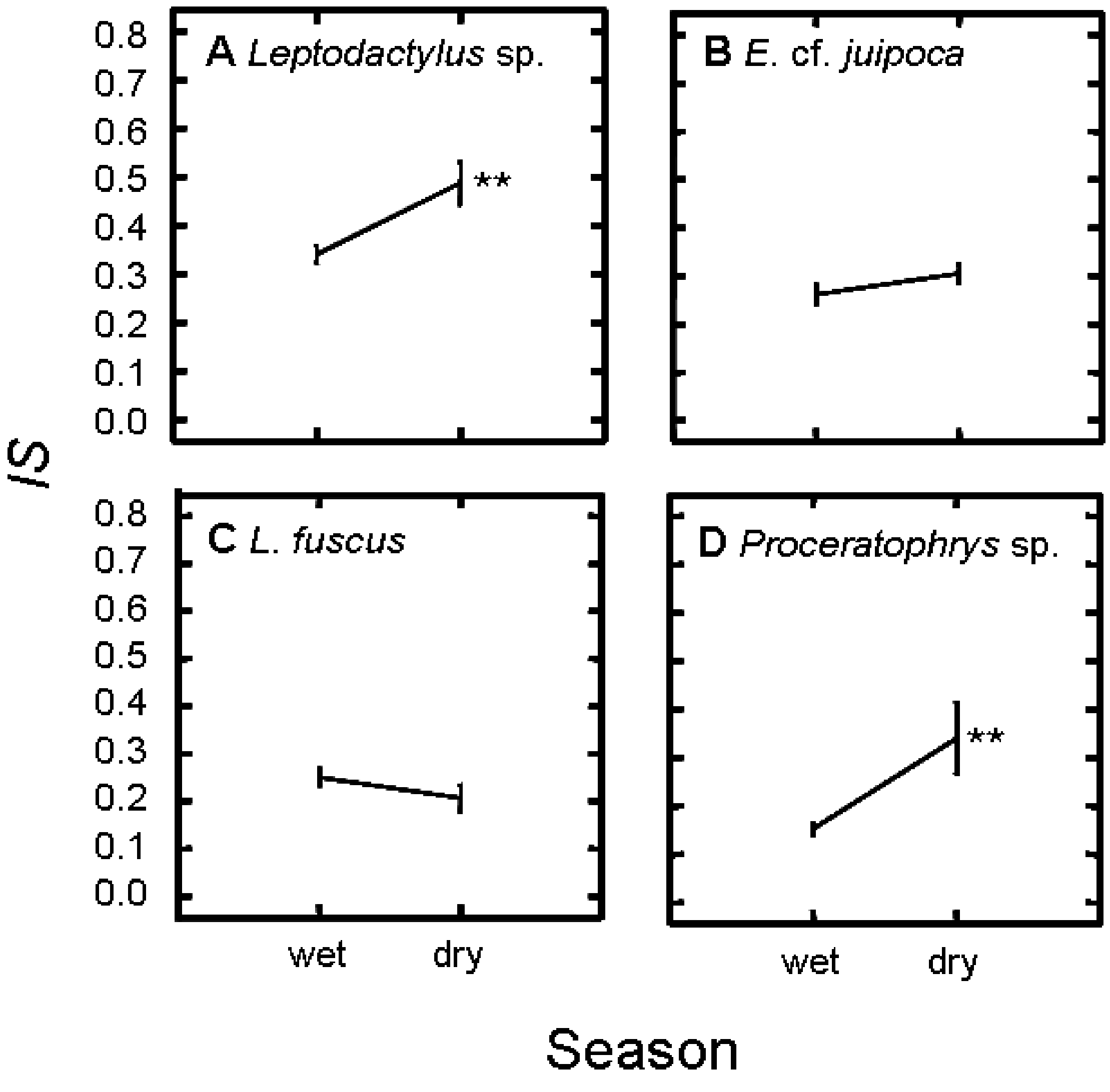

Fig. 1. Comparison between the degree of individual specialization (*IS*; mean ± Standard Error) in the wet and dry seasons in four species of Brazilian frogs. Lower *IS* values indicate stronger individual specialization. A: *Leptodactylus* (= *Adenomera*) sp.; B: *Eleutherodactylus* cf. *juipoca*; C: *Leptodactylus fuscus*; and D: *Proceratophrys* sp. Sample sizes are the same as in Table 2. $^{**}P < 0.01$ (Mann-Whitney *U* test).

The degree of individual specialization was not the same among species (Table 2), being the highest in *Proceratophrys* sp. in the wet season (~ 0.15; recall that values closer to zero indicate stronger individual specialization) and the lowest in *Leptodactylus* sp. in the dry season (~ 0.49; Table 2). A likely explanation for these differences might be the relationship we found between the degree of individual specialization and the population niche width. We found that the broader the population niche the higher the interindividual diet variation. This is in accordance with the patterns previously reported for *Anolis* lizards (Lister 1976; Roughgarden 1979) and experimentally confirmed in three-spine sticklebacks (Svanbäck & Bolnick 2007), suggesting that this may be a general pattern in natural populations. This pattern is expected in the presence of trade-offs, in which individuals are not able to master and consume all resource types used by the population as a whole. In such cases, individual niches remain constrained when the population niche expands, and individual specialization arises as a consequence. Note that this correlation between *TNW* and *IS* is not corrected for phylogenetic non-independence among datapoints. We find this acceptable since individual specialization likely shows substantial variation even among populations and over time as ecological conditions change, so we do not expect a strong phylogenetic signal (Price 1997). Moreover, in our case the use of comparative methods would suffer seriously from the poor taxon sampling and the lack of branch length estimates (Ackerly 2000).

Additionally, seasonality seems to be an important factor in the degree of individual specialization in *Leptodactylus* sp. and *Proceratophrys* sp. (Fig. 1). In both species, we observed a reduction in the degree of individual specialization in the dry season, when there is a great reduction in arthropod abundance in the Cerrado (Pinheiro *et al.* 2002; G. Machado, unpubl.). Individual niche widths may vary over time as a function of resource abundances (Svanbäck & Persson 2004; Svanbäck & Bolnick 2007). Optimal diet theory (Pulliam 1974) predicts that individuals will be more specialized on preferred resources when resources are abundant. As a result, individual niches would tend to shrink in the 'fat' season and expand in the 'lean' season (Schoener 1986). If individuals have distinct preference ranks for resources (see Svanbäck & Bolnick 2005), we might expect a higher degree of individual specialization in the 'fat' season –when individual niches are narrower– and less individual specialization in the 'lean' season –when individual niches are wider and tend to overlap more with the population niche. This expectation is in accordance with the pattern observed in the three species that showed significant or marginally significant seasonal differences in the degree of individual specialization, namely *Leptodactylus* sp., *Proceratophrys* sp., and *E.* cf. *juipoca* (Fig. 1). An alternative explanation relies on the fact that the population niche width increased in the wet season (not shown), when there is a higher diversity of resources available. If individual niche widths remain constant while the population niche expands, the degree of individual specialization will increase. This is in accordance with the idea of population niche expansion through increasing between-individual variation, but in a much faster time scale than the evolutionary diet diversification suggested by Lister (1976) and Roughgarden (1979). This quick intrapopulation diet diversification is more in line with behaviorally based diet shifts (see below) in response to changes in resource abundance, which have been experimentally demonstrated in natural populations of sticklebacks (Svanbäck & Bolnick 2007).

Mechanisms of individual-level diet variation

An important task in the study of individual specialization is to identify its underlying mechanisms (Bolnick *et al.* 2003; Svanbäck & Persson 2004), which in general is associated with the presence of functional trade-offs, either morphological, behavioral or physiological (Bolnick *et al.* 2003). In the case of frogs, an association between morphology and diet is apparent when we compare ant-specialist against generalist species: the former have narrower mouths than the latter (Toft 1980a, 1981). In fact, the specialization in ants seems to result from a combination of morphological, behavioral (active searching vs. sit-and-wait tactic), and physiological (aerobic vs. anaerobic metabolism) characters, and it has been suggested that ant-specialization and generalization correspond to two different adaptive peaks in frogs (Toft 1985). In the present study, all the studied species belong to a guild of generalist feeders (Toft 1981, 1985), and we had no *a priori* reason to expect a correlation between individual morphology and diet. We tested it mainly for two reasons. First, the only way of ruling out morphology as the underlying mechanism of the observed individual-level diet variation was to test morphology against diet. Second, morphology-diet correlations can be a useful way to infer temporal consistency in resource use, which is especially important in the absence of repeated observations of single individuals (see below).

We found no evidence that individual-level diet variation has a morphological basis in the studied frogs, which calls for an alternative explanation. One possible explanation is the existence of learning trade-offs. If resources require learning to be used and individuals are neurologically limited in their learning abilities, learning trade-offs may happen (Werner *et al.* 1981; Lewis 1986; Werner & Sherry 1987; Bernays & Funk

1999). For example, in the Bluegill Sunfish *Lepomis macrochirus*, learning was found to increase foraging efficiency sharply on either benthic (midge larvae) or limnetic prey (*Daphnia*), and was an important factor in determining habitat choice by individuals (Werner *et al.* 1981). It is possible that in the studied frogs, which feed both on highly mobile, elusive prey (e.g., beetles, spiders, roaches) and slow-moving, patchily distributed, prey (e.g., ants, termites), there is some learning involved in prey search, detection, capture, and handling. We acknowledge this is very speculative, but learning trade-offs could be tested experimentally in these frogs by measuring capture rates and handling times of consumers on different prey types (Werner *et al.* 1981; Ehlinger 1990). Another possibility that cannot be dismissed is that frogs differ in their physiological abilities to digest prey, which might also generate trade-offs (Bolnick *et al.* 2003) resulting in individual specialization (West 1986). The studied frogs feed on ants and termites, which are known to be highly toxic prey (Caldwell 1996; Santos *et al.* 2003). These toxins in turn might impose a cost to be detoxified, which may result in trade-offs and generate individual-level diet preferences.

Temporal consistency

Gut contents are a ‘snapshot’ of an individual’s diet and 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 overestimate 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). In a companion study concerning the studied frogs (Araújo et al., in press), we measured the among-individual variance in carbon stable isotopes ($\delta^{13}C$), quantified from the frogs' muscle tissue, as well as their food resources' signatures. Muscle tissue is known to integrate several months of an individual's past food consumption (Tieszen *et al.* 1983; Dalerum & Angerbjörn 2005) and is therefore a useful measure of long-term diet. 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 close to zero (Fry *et al.* 1978). On the other hand, if individuals vary in their isotopic signatures, this can be taken as evidence of long term interindividual diet variation. The studied frogs had isotopic variances ranging from 1.38 in *E.* cf. *juipoca* to 8.35 in *Proceratophrys* sp. (Araújo et al., in press), indicating the existence of among-individual diet variation. Additionally, Araujo et al. (in press) developed a method to convert this variance into the *IS* index of individual specialization. By comparing the isotope-derived *IS* measures with those obtained from gut contents, these authors demonstrated that gut contents only slightly overestimated individual-level diet variation in *Leptodactylus* sp., *L. fuscus*, and *Proceratophrys* sp., while greatly overestimating the degree of individual specialization in *E.* cf. *juipoca*. Bearing this caveat in mind, we feel confident to state that with the exception of *E.* cf. *juipoca*, whose isotope-derived *IS* measure was around 0.8, there is evidence of strong individual-level diet variation in the studied species.

Conclusions

We have documented the first cases of individual specialization in tropical, diverse communities of frogs. Our results are at odds with the general pattern of competitive release-driven niche expansion in depauperate communities, leading to increased intra-population variation described in the ecological literature (e.g., Roughgarden 1974; Werner & Sherry 1987; Smith & Skúlason 1996). The observed pattern is particularly striking since the actual degree of individual specialization observed in these tropical frogs is comparable to many of the most ecologically variable populations described from temperate regions (e.g., *Nucella* snails; West, 1986, 1988; threespine sticklebacks; Svanbäck and Bolnick, 2007), and depauperate tropical habitats (e.g., Cocos Finches; Werner and Sherry, 1987). While we have not statistically compared levels of diet variation in these tropical frogs to temperate taxa (there are too few comparable studies for a robust test), our results clearly indicate that individual specialization does occur in diverse communities. The description of this new pattern, by challenging an established view, may promote a better understanding of the necessary conditions for the evolution and maintenance of individual specialization as well as its implications for species coexistence.

MATERIAL EXAMINED

Leptodactylus sp.—Brazil: Minas Gerais: Uberlândia, Clube de Caça e Pesca Itororó de Uberlândia, 850 m, AAG-UFU 3742-60.

E. cf. *juipoca*.—Brazil: Minas Gerais: Uberlândia, Estação Ecológica do Panga, 850 m, AAG-UFU 4122-4.

Leptodactylus fuscus.—Brazil: Minas Gerais: Uberlândia, Clube de Caça e Pesca Itororó de Uberlândia, 850 m, AAG-UFU 3931-4.

Proceratophrys sp.—Brazil: Minas Gerais: Uberlândia, Clube de Caça e Pesca Itororó de Uberlândia, 850 m, AAG-UFU 3817-45.

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APPENDIX 1

Diet composition of *Leptodactylus* sp. (*n* = 100), *Eleutherodactylus* cf. *juipoca* (*n* = 107), *L. fuscus* (*n* = 83), and *Proceratophrys* sp. (*n* = 50) in the Brazilian Cerrado, represented as the number of prey items consumed and their proportions (in parenthesis) in each species. [*]Categories included in the analyses (see text for details).

Prey categories	Species			
	Leptodactylus sp.	*E.* cf. *juipoca*	*L. fuscus*	*Proceratophrys* sp.
Collembola	6 (0.01)	49 (0.11)[*]	0	0
Odonata (naiads)	0	0	0	1 (0.01)
Dermaptera	3 (0.00)	0	2 (0.00)	1 (0.01)
Orthoptera (NI)	2 (0.00)	7 (0.02)	12 (0.03)[*]	7 (0.04)
Orthoptera (soil)[a]	4 (0.01)	6 (0.01)	27 (0.06)[*]	4 (0.02)
Orthoptera (vegetation)[b]	2 (0.00)	4 (0.01)	14 (0.03)[*]	6 (0.03)[*]
Blattodea	44 (0.07)[*]	13 (0.03)[*]	22 (0.05)[*]	16 (0.09)
Mantodea	0	1 (0.00)	2 (0.00)	0
Isoptera (NI)	2 (0.00)	5 (0.01)	1 (0.00)	0
Isoptera (alates)	24 (0.04)[*]	5 (0.01)	13 (0.03)[*]	9 (0.05)
Isoptera (non-alates)	31 (0.05)[*]	0	53 (0.12)[*]	0
Psocoptera	0	21 (0.05)[*]	0	0
Thysanoptera	0	1 (0.00)	0	0
Hemiptera				
Auchenorrhinca[c]	34 (0.06)[*]	41 (0.09)[*]	22 (0.05)[*]	9 (0.05)
Sternorrhinca[d]	1 (0.00)	4 (0.01)	1 (0.00)	4 (0.02)
Heteroptera (NI)	14 (0.02)	1 (0.00)	7 (0.02)	1 (0.01)

Heteroptera (aquatic)[e]	0	0	1 (0.00)	0
Heteroptera (soil)[f]	2 (0.00)	1 (0.00)	3 (0.01)	2 (0.01)
Heteroptera (vegetation)[g]	9 (0.01)	6 (0.01)	3 (0.01)	10 (0.05)
Coleoptera (NI)	15 (0.02)	12 (0.03)*	14 (0.03)*	5 (0.03)
Coleoptera (soil)[h]	36 (0.06)*	23 (0.05)*	26 (0.06)*	4 (0.02)
Coleoptera (vegetation)[i]	12 (0.02)	8 (0.02)	36 (0.08)*	8 (0.04)
Lepidoptera	0	1 (0.00)	2 (0.00)	0
Trichoptera	0	1 (0.00)	0	0
Diptera (NI)	0	0	7 (0.02)	0
Diptera (Nematocera)	7 (0.01)	6 (0.01)	3 (0.01)	2 (0.01)
Diptera (other)[j]	7 (0.01)	7 (0.02)	6 (0.01)	2 (0.01)
Hymenoptera[k]	6 (0.01)	7 (0.02)	3 (0.01)	2 (0.01)
Formicidae	149 (0.25)*	101 (0.22)*	79 (0.18)*	13 (0.07)
Insect larvae (aquatic)[l]	11 (0.02)	3 (0.01)	3 (0.01)	1 (0.01)
Insect larvae (terrestrial)[m]	64 (0.11)*	8 (0.02)	17 (0.04)*	16 (0.09)
Chilopoda	1 (0.00)	12 (0.03)*	4 (0.01)	5 (0.03)
Diplopoda	10 (0.02)	0	0	5 (0.03)
Isopoda	4 (0.01)	0	1 (0.00)	11 (0.06)
Scorpiones	0	0	1 (0.00)	2 (0.01)
Pseudoscorpiones	4 (0.01)	0	0	0
Acari	6 (0.01)	1 (0.00)	9 (0.02)	0
Opiliones	4 (0.01)	6 (0.01)	1 (0.00)	6 (0.03)
Araneae (NI)	19 (0.03)*	20 (0.04)*	7 (0.02)	5 (0.03)

Araneae (soil)[n]	18 (0.03)*	20 (0.04)*	11 (0.02)	5 (0.03)
Araneae (vegetation)[o]	12 (0.02)	24 (0.05)*	8 (0.02)	2 (0.01)
Araneae (both)[p]	14 (0.02)	21 (0.05)*	5 (0.01)	3 (0.02)
Gastropoda	8 (0.01)	7 (0.02)	2 (0.00)	2 (0.01)
Oligochaeta	4 (0.01)	0	2 (0.00)	1 (0.01)
Anura	0	0	1 (0.00)	0
Seeds	12 (0.02)	0	13 (0.03)*	12 (0.07)
Total	601	453	444	182

NI: non-identified; a: Gryllacrididae, Gryllidae, Gryllotalpidae, Tridactylidae; b: Acrididae, Tettigoniidae; c: Cicadidae, Cercopidae, Membracidae, Cicadellidae; d: Aphididae, Coccidae, Psyllidae; e: Veliidae; f: Cydnidae, Gelastocoridae; g: Berytidae, Reduviidae, Ploiariidae, Coreidae, Lygaeidae, Pyrrhocoridae, Rhopalidae, Pentatomidae, Miridae, Tingidae; h: Scarabaeidae, Tenebrionidae, Carabidae, Staphylinidae, Limulodidae, Pselaphidae, Nitidulidae; i: Chrysomelidae; Cerambycidae; Coccinelidae; Elateridae; Cantharidae; Lampyridae; Bostrichidae; Scolytidae; Endomychidae; j: Brachycera, Cyclorrhapha; k: Chalcidoidea; l: Dytiscidae; m: Coleoptera, Lepidoptera, Elateridae, Diptera, Neuroptera; n: Actinopodidae, Theraphosidae, Ctenidae, Lycosidae, Oonopidae, Caponidae, Corinnidae; o: Thomisidae, Mimetidae, Salticidae, Oxyopidae, Philodromidae, Anyphaenidae, Dictynidae, Araneidae, Scytodidae; p: Linyphiidae, Theridiidae, Pholcidae, Pisauridae, Miturgidae.

Capítulo 2

Using $\delta^{13}C$ stable isotopes to quantify individual-level diet variation
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Using $\delta^{13}C$ Stable Isotopes to Quantify Individual-Level Diet Variation

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ABSTRACT

Individual-level diet variation can be easily quantified by gut-content analysis. However, because gut contents are a 'snapshot' of individuals' feeding habits, such cross-sectional data can be subject to sampling error and lead one to overestimate levels of diet variation. In contrast, stable isotopes reflect an individual's long-term diet, so isotope variation among individuals can be interpreted as diet variation. Nevertheless, population isotope variances alone cannot be directly compared among populations, because they depend on both the level of diet variation and the variance of prey isotope ratios. We developed a method to convert population isotope variances into a standardized index of individual specialization (*WIC/TNW*) that can be compared among populations, or to gut content variation. We applied this method to diet and carbon isotope data of four species of frogs of the Brazilian savannah. Isotopes showed that gut contents provided a reliable measure of diet variation in three populations, but greatly overestimated diet variation in another population. Our method is sensitive to incomplete sampling of the prey and to among-individual variance in fractionation. Therefore, thorough sampling of prey and estimates of fractionation variance are desirable. Otherwise, the method is straightforward and provides a new tool for quantifying individual-level diet variation in natural populations that combines both gut-content and isotope data.

Key-words: carbon stable isotopes; *Cerrado*; gut contents; individual specialization; fractionation

INTRODUCTION

Many natural populations are composed of ecologically heterogeneous individuals that use different subsets of the available resources (Van Valen 1965; Roughgarden 1972; Heinrich 1979; Price 1987; Werner & Sherry 1987; Svanbäck & Bolnick 2005). Individuals within a population may use different resources because they inhabit different microhabitats (Durell 2000), are different sexes (Slatkin 1984), or different ages (Polis 1984). However, individuals can also exhibit niche variation within sex or age-class, and within a single site or time. This individual-level variation is called "individual specialization", in which individuals use a significantly narrower set of resources than the population as a whole (Bolnick et al. 2003). This variation may have important ecological implications, such as a reduction of intraspecific competition (Swanson et al. 2003) or the differential response of individuals to both intra and interspecific competition (Taper & Case 1985) or predation, which can ultimately affect population dynamics (Lomnicki 1992). Moreover, this variation permits frequency-dependent interactions that can drive disruptive selection and evolutionary divergence (Dieckmann & Doebeli 1999; Bolnick 2004).

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 *et al.* 1998; Svanback and Bolnick, manuscript). An important underlying assumption in these studies is that the prey found in stomachs actually represent the long-term resource use of individuals. However, this assumption may not hold if prey are patchily distributed, their abundances vary over time, or stomachs can only contain a few items at a time, because individuals' gut contents reflect their recent encounters rather than long-term preferences. For instance, Warburton et al. (1998) analyzed the gut contents of the silver perch (*Bidyanus bidyanus*) and observed that individuals were highly specialized on different resources, but only over periods of time of 2-4 weeks, after which they changed their diets in response to prey abundance variation. These sampling problems will lead one to believe individuals are more specialized than they really are, overestimating the degree of diet variation (Bolnick *et al.* 2002; Bolnick *et al.* 2003).

There are cases, however, in which gut contents are a fairly good indicator of individual long-term resource use. The most compelling examples come from studies on fishes, in which researchers repeatedly sampled stomachs of the same individuals and observed high temporal consistency of individual diets (e.g. Bryan & Larkin 1972; Schindler 1997). Other studies showed that morphological variation among consumers explained some of the variation in stomach contents (Robinson *et al.* 1993; Svanback and Bolnick manuscript; Fermon & Cibert 1998). Such morphology-diet correlations are strong evidence that some of the stomach content variation represents consistent diet variation among foragers. Finally, several studies have relied on the quantification of stable isotopes (Fry et al. 1978; Gu et al. 1997) to infer temporal consistency in the diets of individuals.

The utility of stable isotopes in diet studies is that the sources of for example carbon and nitrogen can be distinguished so that a consumer's diet can be inferred. Since stable isotopes have relatively slow turnover rates compared to feeding episodes, varying from days to years depending on the tissue analyzed (several months in the case of muscle), they can be used to infer dietary carbon and nitrogen intake over long time periods (Tieszen *et al.* 1983; Dalerum & Angerbjörn 2005). 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 food-resource use. In fact, carbon stable isotopes have been used as a measure of intra-population diet variation (Fry *et al.* 1978; Angerbjörn *et al.* 1994; Gu *et al.* 1997; Sweeting *et al.* 2005). For example, Fry et al. (1978) measured the standard deviation (SD) of individual carbon isotope ratios in different species of grasshoppers and observed that species that fed on both C_3 and C_4 plants had higher SD values than those specialized on either C_3 or C_4 plants. Isotope variation thus offers a method of testing for diet variation that is complementary to gut-content analysis, and can be used to evaluate the reliability of gut contents.

However, using isotope variance to test for individual specialization has some important caveats. If there are more food sources than we can discriminate with isotopes (Phillips & Gregg 2003), isotope variation may underestimate diet variation among individuals (Matthews & Mazumder 2004). On the other hand, if food sources show isotopic variation in space and/or time and consumers were sampled in different places or times, one will observe isotopic variation that is not necessarily related to diet variation (Dalerum & Angerbjörn 2005; Matthews & Mazumder 2005). Moreover, for a given level of diet variation, populations using more isotopically variable prey will themselves show higher isotope variances (Matthews & Mazumder 2004). Consequently, measures of population isotopic variance can be a misleading guide diet variation if the prey isotopic variance is not taken into account. Matthews and Mazumder (2004) proposed null models that allow one to test for significant individual specialization in populations, provided that the isotope ratios of prey are known. Their method, therefore, allows one to test the null hypothesis that individuals in a population sample randomly from the population distribution (individual generalists), and as a consequence to detect cases of individual specialization. However, they do not allow us to use isotope variances to quantify the degree of individual specialization or compare the amount of diet variation among populations. Therefore, it would be useful to be able to scale isotopic variance to a measure of individual specialization that can be compared across different populations.

In this paper, we present a method that allows the use of $\delta^{13}C$ variance to estimate a standardized index of individual specialization (Bolnick et al. 2002) that can be compared across different populations. We apply this method to isotope and gut-content data of four populations of leptodactylid frogs (*Adenomera* sp., *Eleutherodactylus* sp., *Leptodactylus fuscus*, and *Proceratophrys* sp.) that inhabit an area of savannah in southeastern Brazil. These are terrestrial, relatively sedentary animals, feeding in a potentially patchy environment, in which we would expect gut contents to overestimate diet variation due to stochasticity in food consumption. By comparing the estimates of the degree of individual specialization resulting from our method to those derived from gut contents, we were able to evaluate the utility of cross-sectional data in studies of diet variation.

MATERIALS AND METHODS

Study area

We analyzed the stomach contents and stable carbon isotopes of muscle tissue of four species of frogs from a savannah formation in southeastern 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 et al. 1991). Specimens of four species (*Adenomera* sp., *Eleutherodactylus* sp., *Leptodactylus fuscus*, and *Proceratoprhys* sp.; *N* = 104, 115, 86, and 55 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 (18º 55' S - 48º 17' W, 850 m), in the state of Minas Gerais, southeastern Brazil, in five sites within each of two of the remnants of *cerrado* still present in the municipality (Goodland & Ferri 1979). Frogs were collected weekly in the wet season and once every two weeks in the dry season, for a period of two years. Frogs were fixed in 5% formalin and later preserved in 70% ethanol.

Data collection

Diet data

Preserved specimens were dissected under a microscope to obtain stomach contents. Upon dissection individuals were sexed by examination of gonads. Prey items were counted, measured for total length using an eyepiece coupled with a stereomicroscope, and identified to order or more commonly family level (following Borror & DeLong 1988).

Stable isotopes

We measured stable isotopes from the frogs and the prey found in gut contents. Carbon isotopic signatures of animal tissues can be altered by ethanol and formalin preservation (Kaehler & Pakhomov 2001; Sweeting *et al.* 2004). However, since we are interested in estimating the variance among individual isotopic ratios and all our samples were subject to the same preservation conditions, preservation should not be a problem in our study. To quantify $\delta^{13}C$ in the frogs, a piece of muscle from the thigh was collected from a subsample of 60 specimens chosen randomly from the larger sample of available specimens (*Adenomera* sp., *Eleutherodactylus* sp., and *L. fuscus*); in the case of *Proceratophrys* sp. all the 55 individuals were analyzed. To quantify $\delta^{13}C$ in the prey, we analyzed whole prey items obtained from 47 gut contents across the four species. Some prey taxa were not abundant or large enough to measure isotopic ratios. Those prey were all very rare in the samples, each one representing no more than 1% of the number of prey consumed, and were lumped under the category 'Others' (see Electronic Supplementary Material 1 for details). Samples were rinsed for one minute in distilled deionised water (Sweeting et al. 2004), oven-dried to constant mass at 50°C (Magnusson et al. 1999), ground, and weighed (c. 1mg) into 4x3.2mm tin capsules. Prey items were grouped by taxon, generally Order, though we split Coleoptera and Heteroptera into finer categories based on feeding habits (according to Borror & DeLong 1988). We did this to minimize isotope variation within taxa. A list of the taxa comprising each of these feeding-habit groups is provided in Table S1 (Electronic Supplementary Material 1). We analyzed the isotopes of a total of 23 prey categories (Table S1). Prey items belonging to the same categories were dried and ground together. The abundances of ^{13}C and ^{12}C were determined at the University of California at Davis Stable Isotope Facility using a continuous flow, isotope ratio mass spectrometer (CF-IRMS, Europa Scientific, Crewe, UK), interfaced with a CN sample converter. Two samples of an internal reference material were analyzed after every 12 samples, to calibrate the system and to compensate for drift with time. The $^{13}C/^{12}C$ compositions are reported using conventional delta notation, showing differences between the observed concentration and that of Pee Dee Belemnite. Experimental precision was estimated as the standard deviation of replicates of the internal reference material, and was 0.03‰.

Prey dry masses

We estimated the dry masses of prey categories by weighing all the remaining intact items found in the stomach contents. Items belonging to each category were oven-dried and weighed in a high-precision balance (0.01mg). The final dry mass of each category was divided by the number of items weighed, which varied from 2 to 202 ($\bar{x} = 37.2$; SD = 51.5). There were four categories (Coleoptera Dead-Wood Consumers; Heteroptera Granivores; Heteroptera Predators; and Hymenoptera Non-Formicidae) for which we were not able to directly estimate dry masses due to insufficient material. In those cases, we used published regression equations (Sample *et al.* 1992; Hódar 1996) to estimate insect biomasses from length measures.

Data analyses

Analysis of diet data

Diet variation can be a function of sex or age. Moreover, both diet and isotopic variation are subject to spatial and temporal effects. As a consequence, we had to rule out these confounding effects before quantifying diet and isotopic variation in our samples.

We therefore tested if the diets and isotopic signatures among individuals varied as a function of sex, age class, collection site, and season (wet and dry). In the case of the diet data, we first did a Principal Component Analysis on the arcsine-square root transformed proportions of prey use by individuals. We then took the PC-scores of the major axes (axes that explained > 5% of the variation) and did a multi-way MANOVA, with PC-scores as dependent variables and sex, age class, collection site, and season as factors. In addition, we used a multi-way ANOVA to test for those same effects on $\delta^{13}C$ ratios within each frog species. By doing this, we ensure that any diet or isotope variation is not due to either sex/age effects or spatial/temporal variation in prey availability or signatures. This in turn allows us to interpret the results in terms of individual specialization. The MANOVAs and ANOVAs were performed in SYSTAT11.

We next calculated a measure of the degree of individual specialization using frequencies of prey categories in individuals' guts. We used Roughgarden's (1979) measure of individual specialization *WIC/TNW*, in which *TNW* is the total niche width of a population, *WIC* is the within-individual component of niche width (average of individual niche widths), and *BIC* is the between-individual component (the variance among individuals' niches). Traditionally, the degree of diet variation is described by calculating the percent of total niche variation ascribed to individual niche widths (*WIC/TNW*). The higher the value of *WIC* relative to *TNW*, the less variable individuals are, and vice-versa. Therefore, *WIC/TNW* varies from 0 (maximum variation among individuals) to 1 (no variation among individuals). For comparison, we also estimated a second measure of individual specialization (*IS*) based on distribution-overlap (Bolnick et al. 2002). Since results were qualitatively the same, we focus on the former measure.

Readers are referred to Bolnick et al. (2002) for the formulas of the indices and details on their calculation. All the analyses of individual specialization were performed in IndSpec1, a program to calculate indices of individual specialization (Bolnick et al. 2002).

We used a nonparametric Monte Carlo procedure to test the null hypothesis that any observed diet variation arose from individuals sampling stochastically from a shared distribution. Each individual was randomly reassigned a new diet via multinomial sampling from the observed population resource distribution, and *WIC/TNW* was recalculated for the resulting population diet variation. IndSpec1 generated 1,000 such populations, and the null hypothesis can be rejected if the observed value of *WIC/TNW* is less than 95% of the null *WIC/TNW* values. This Monte Carlo procedure assumes that every prey item observed in a stomach represents an independent feeding event. We acknowledge, however, that this assumption may be violated for prey such as ants and termites that are found in tightly clustered groups.

Comparing isotope variation to gut-content variation

We developed a method that allows us to quantify the degree of individual specialization based on among-individual isotope variation (Figure 1). This method uses the observed population diet to generate a large number of simulated populations with varying degrees of individual specialization ($0 < WIC/TNW < 1$). Empirical prey isotope ratios and dry masses are then used to calculate the isotopic variance Varδ_i for each simulated population. The simulations thus establish a relationship between Varδ_i and *WIC/TNW*. Finally, we use this relationship to convert an empirical Varδ_i into an estimate of *WIC/TNW* (Fig. 1). In our model, we are making three important simplifications: first that individuals do not selectively assimilate different isotopic components of a food source (differential assimilation); second that there is no fractionation between a consumer and its diet; and third that there is no isotopic routing (Gannes et al. 1997). We acknowledge, however, that these processes can potentially affect the estimates of a population's isotopic variance and consequently the interpretations of our model. We address this problem further in the Discussion. In the following paragraphs, we explain in detail how population diets and $\text{Var}\delta_i$ were simulated.

Each simulated population was composed of the empirically observed number of individuals, *N*. Each individual's resource distribution was assigned by a multinomial sample from the empirical population's resource distribution. We could control the level of diet variation among individuals by setting the number of multinomial draws that each individual took from the population's distribution. Due to the Law of Large Numbers, individuals given few draws had narrower and, as a consequence, more variable diets than when individuals had many draws. We would like to emphasize that this approach is merely a technique to generate different levels of among-individual diet variation and does not assume any underlying biological mechanism.

The first step in our simulation is to sum across the stomach contents of all *N* consumers in our empirical sample and calculate the frequency $p_{\bullet j}$ of each diet type *j* in the overall population's resource distribution. The resulting population diet vector is $\mathbf{p}_{\bullet} = (p_{\bullet 1}, p_{\bullet 2}, p_{\bullet 3}, \ldots p_{\bullet k})$. Then, each simulated individual is given *s* random draws (with

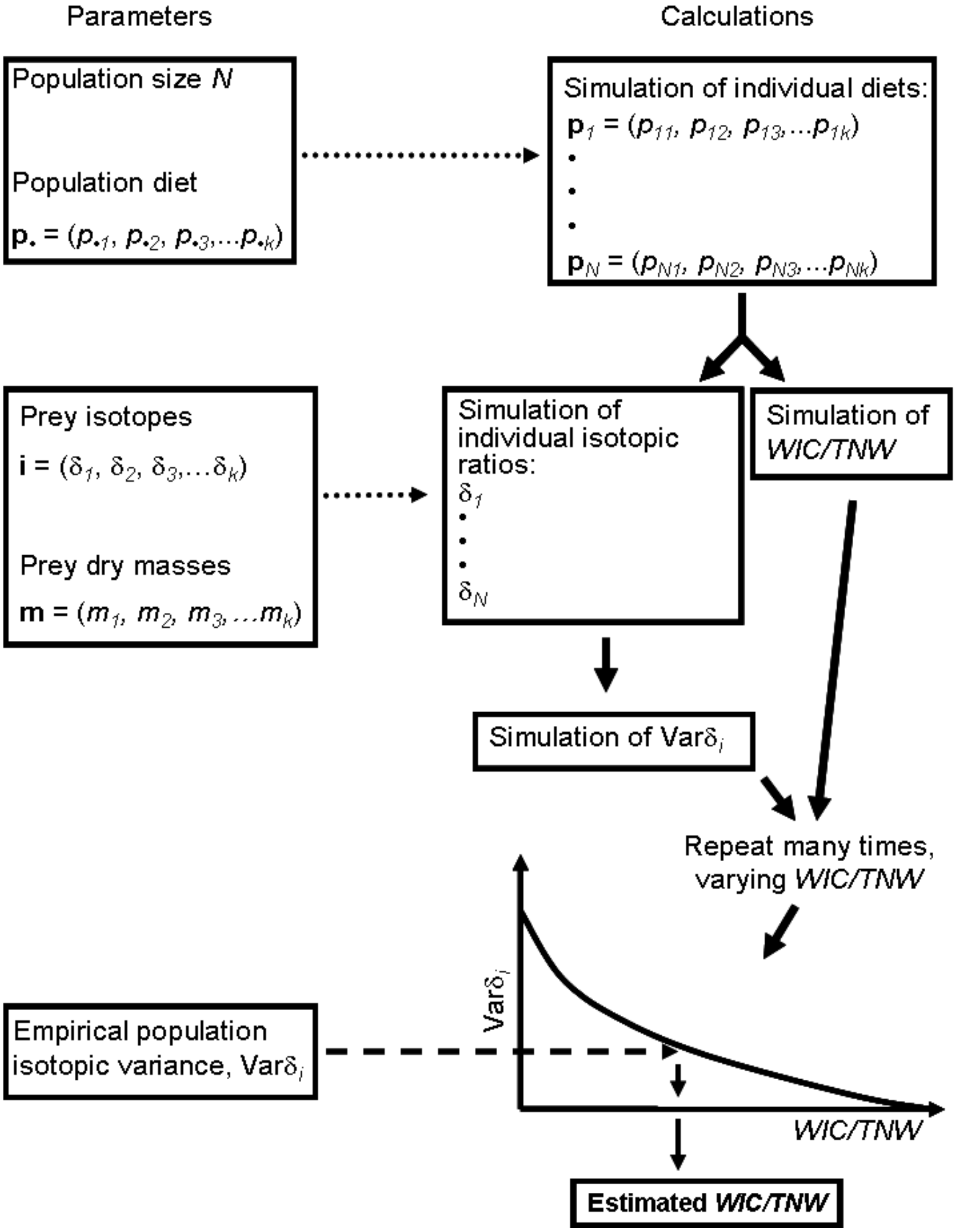

Fig. 1. Flow chart of the model used to generate measures of individual diet specialization (*WIC/TNW*) and among-individual variance in isotopic ratios (Varδ_i) of simulated populations. The chart outlines the procedure to generate simulated populations, showing parameters on the left and calculations on the right, composed of *N* individuals feeding on *k* prey categories with δ^{13}C signatures δ_k and dry mass m_k, and calculations to interpolate an estimated *WIC/TNW* from the empirical prey isotope variance. See text for details.

replacement) from this multinomial probability distribution. The goal is to use the resulting number of draws (n_{ij}) of each prey type j to represent a long-term diet vector $\mathbf{p}_i$ for the simulated individual (Fig. 1). Although we acquired this vector by a sampling process, we use it to represent the vector of individual diet preferences. If an individual is given only a single draw (s = 1), it will persistently specialize on a single type of prey resource, e.g., $\mathbf{p}_i$ = (1.0, 0, 0…0). Since different individuals will eventually draw different prey from the population vector, s = 1 yields the maximum level of among-individual variation. As s increases, individuals' diet vectors $\mathbf{p}_i$ converge towards $\mathbf{p}_{\bullet}$ (Law of Large Numbers) and diet variation declines.

After calculating the $\mathbf{p}_i$ vectors, our simulation uses the empirically-obtained prey masses and isotope signatures to calculate each simulated individual's isotope signature

$$E(\delta_i) = \sum_j \frac{p_{ij} m_j}{\sum_j p_{ij} m_j} \delta_j$$

The program then calculates *WIC/TNW* and the population isotopic variance Varδ_i, which are the outputs for the simulated population (Fig. 1). The model repeats this procedure for n replicate populations for each of 57 values of s (ranging from 1 to 1,000 in increasing increments). In our simulations, n was set at 100. A PC-compatible program, VarIso1, to perform these simulations was written in C language and is available for public use at http://webspace.utexas.edu/dib73/Bolnicklab/links.htm.

We used quadratic regressions to establish the relationship between simulated *WIC/TNW*, and Varδ_i. We used the resulting equation, and the empirical value of Varδ_i, to solve for an estimated value of *WIC/TNW* (Fig. 1). Confidence intervals were obtained using a prediction interval (the limits within which a new observation would lie if added to the regression model, with a probability of 95%), obtained in STATISTICA6.0. Finally, we tested whether *WIC/TNW* values from stomach contents fell outside the confidence interval for the isotope-derived value, which would indicate that stomach contents are a poor guide to long-term diet variation.

RESULTS

Diet and stable isotopes data

All four frog species are generalist, feeding on a wide range of prey categories (Table S1). However, any given individual's stomach contained only a subset of its population's resource distribution, so that *WIC/TNW* < 0.5 for all four species (Table 1). This means that within-individual variation only accounted for ~40 – 50% of the total niche width, ranking among the strongest measures of individual specialization in the published literature (Bolnick et al 2003). This diet variation is greater than would be expected under random independent sampling of prey from a common distribution (Monte Carlo bootstraps; Table 1). However, as discussed above, gut contents may not be a reliable measure of diet variation. Turning instead to isotope data, we found that isotope variances ranged from 1.38 (*Eleutherodactylus* sp.; Table 1; Fig. 2b) to 8.35 (*Proceratophrys* sp.; Table 1; Fig. 2c). Prey isotopic signatures were also variable, spanning from -24.57 to -13.32‰ (Table S1; Figure 2).

Sex, age, and season had no significant effects on gut contents or isotopes (Table S2). This indicates that resource use differences were not an artifact of collection season, and that diet variation occurred at the individual level. We therefore pooled samples by sex, age, and date in later analyses. However, we did observe an effect of collection site on isotope ratios (*Adenomera* sp. and *L. fuscus*; Table S2) and gut contents

(*Proceratophrys* sp.; Table S2). In those cases, we did additional post-hoc tests (Tukey) in order to identify those sites that differed from each other. Based on these results (not shown), we split samples of *Adenomera* sp. and *L. fuscus* into two subsamples (henceforth ss1 and ss2). In the case of *Proceratophrys* sp., we removed the sparsely collected site 1 ($N = 3$ frogs) from the analyses.

Table 1. Measures of intra-population variation in food-resource use in four species of Brazilian frogs. WIC/TNW_{obs}: Roughgarden's (1979) index of individual specialization based on gut-content data; $\text{Var}\delta^{13}\text{C}$: empirically estimated isotopic variances of frog samples; WIC/TNW_{exp}: expected value of the index based on isotope data. Numbers in parenthesis are sample sizes. *L. fuscus*: *Leptodactylus fuscus*. Empirical *WIC/TNW* values were tested against null distributions generated with Monte Carlo bootstraps (1,000 simulations); $^{**}P = 0.01$; $^{***}P < 0.001$. ss1 and ss2: subsamples 1 and 2 respectively (see text for details).

Species	WIC/TNW_{obs}	$\text{Var}\delta^{13}\text{C}$	WIC/TNW_{exp}
Adenomera sp.			
ss1 (39)	0.4738^{***}	5.38	0.3967
ss2 (35)	0.4266^{***}	4.90	0.4155
Eleutherodactylus sp. (56)	0.4573^{**}	1.38	0.8636
Proceratophrys sp. (49)	0.3700^{***}	8.35	0.1585
L. fuscus			
ss1 (38)	0.4873^{***}	2.87	0.4965
ss2 (29)	0.4127^{***}	2.01	0.6419

Comparing isotope variation to gut-content variation

Our simulations provided an expected relationship between *WIC/TNW*, and $\text{Var}\delta_i$ (Fig. 3). Using the empirical $\delta^{13}\text{C}$ variances and this curvilinear relationship, we estimated values of *WIC/TNW* (Fig. 3). The values of *WIC/TNW* obtained from gut contents consistently fell within the isotope-derived confidence intervals in *Adenomera* sp. and in *L. fuscus*-ss1, but outside the confidence intervals in *Eleutherodactylus* sp., *L. fuscus*-ss2, and *Proceratophrys* sp. (Fig. 3). Contrary to our expectations, stable isotopes indicated that individual specialization was actually stronger than we inferred from gut contents in *Proceratophrys* sp. (Fig. 3d). In *L. fuscus*-ss2 (Fig. 3f), gut contents revealed a higher level of individual specialization than did the isotopes. Isotopes revealed negligible diet variation in *Eleutherodactylus* sp. (Fig. 3c), in stark contrast to the gut content results. Results using the *IS* index of individual specialization were qualitatively similar (Table S3, Fig. S1).

DISCUSSION

Our results show that there is evidence of individual specialization in the studied populations, and that species vary in the degree of individual specialization. Surprisingly, gut-content variation provided fairly good estimates of overall levels of individual specialization in *Adenomera* sp. and *L. fuscus*. On the other hand, gut contents greatly overestimated individual specialization in *Eleutherodactylus* sp., and greatly underestimated it in *Proceratophrys* sp. (Fig. 3). In the following discussion, we comment on: 1) why gut contents and isotopes may over- or under-estimate individual specialization; 2) the impact of missing prey categories; and 3) the impact of the variance in fractionation among individuals on our method.

Value of gut contents and isotopes in measuring diet variation

Had we only analyzed gut contents, we would have concluded that the four species had roughly similar degrees of individual specialization (~ 0.45). Double-checking these estimates with comparable isotope-derived measures of diet variation, we found a moderately close agreement between gut-content and isotope-based measures of individual specialization in *Adenomera* sp. and *L. fuscus*. This supports the idea that gut-content variation may be a reasonable measure of diet variation in some systems, even in

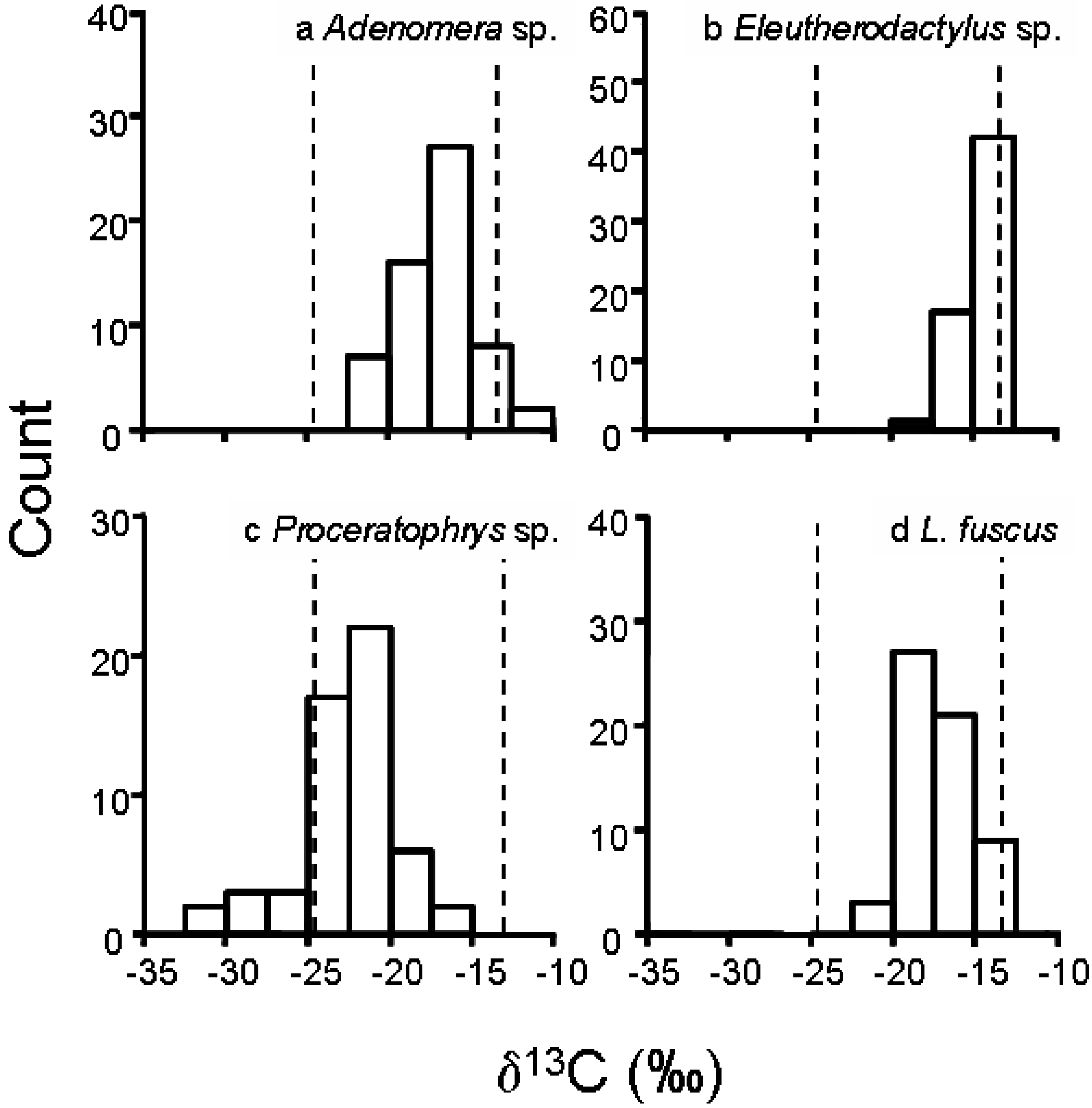

Fig. 2. Histograms of the empirically measured individual $\delta^{13}C$ signatures in four species of Brazilian frogs: **a** *Adenomera* sp. (*N* = 60); **b** *Eleutherodactylus* sp. (*N* = 60); **c** *Proceratophrys* sp. (*N* = 55); **d** *Leptodactylus fuscus* (*N* = 60). Dashed lines indicate the range of $\delta^{13}C$ of consumed prey.

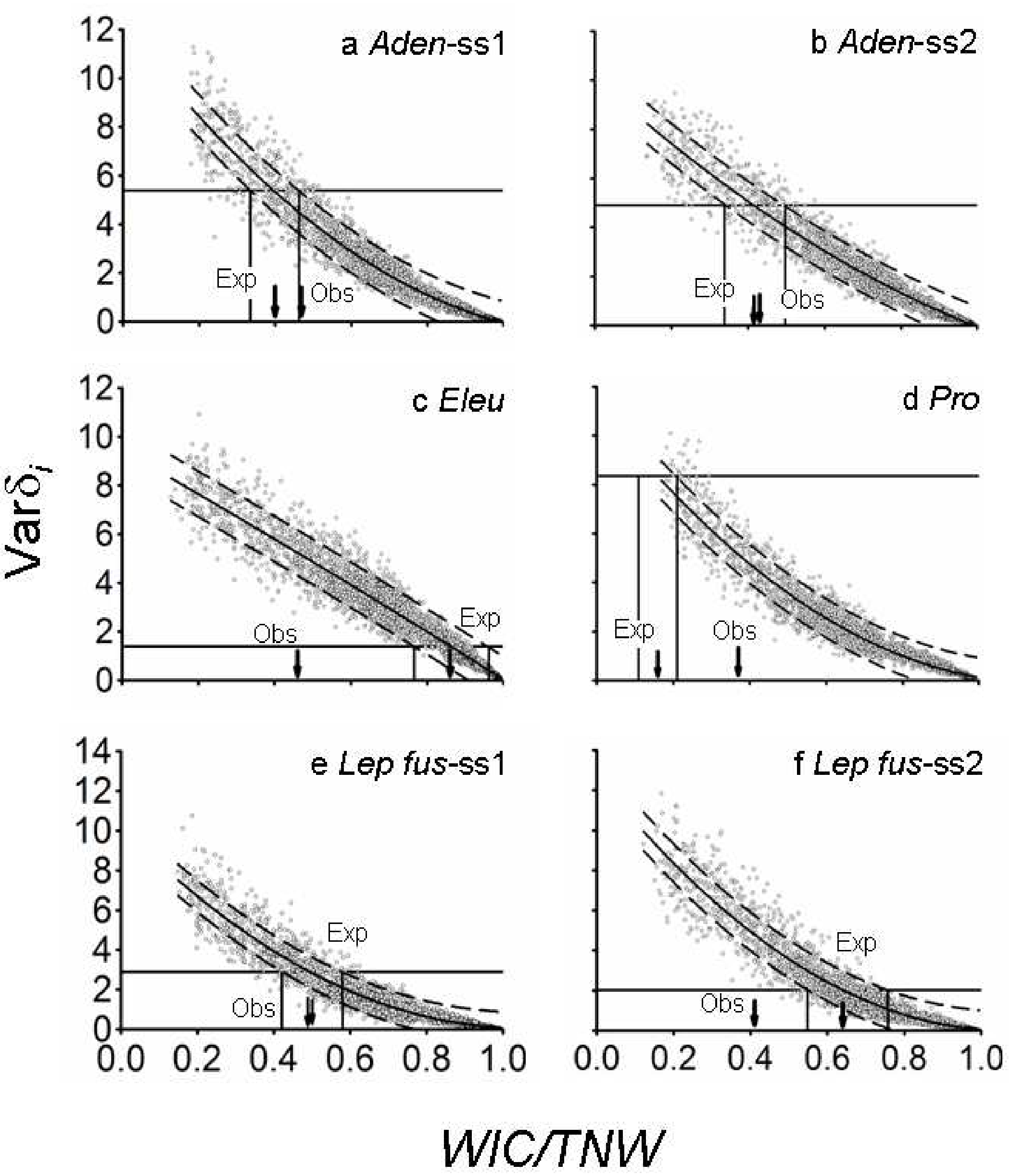

Fig. 3. Interpolation of *WIC/TNW* from isotope variances: the values of δ^{13}C variances (Varδ_i) were regressed onto measures of individual specialization (*WIC/TNW*) of simulated populations (see text for details). Solid curves indicate quadratic fitted regressions; dashed curves are the prediction bands of the regressions; horizontal solid lines indicate the empirically estimated Varδ_i; vertical solid lines define the confidence limits (95%) around the expected *WIC/TNW*. Arrows indicate the expected (Exp) *WIC/TNW* interpolated from the empirical Varδ_i using the regression equations, and the observed (Obs) *WIC/TNW* from gut contents of four Brazilian frogs. **a** *Adenomera* sp.-ss1 (N = 39); **b** *Adenomera* sp.-ss2 (N = 35); **c** *Eleutherodactylus* sp. (N = 56); **d** *Proceratophrys* sp. (N = 55); **e** *Leptodactylus fuscus*-ss1 (N = 38); **f** *Leptodactylus fuscus*-ss2 (N = 29). ss1 and ss2: subsamples 1 and 2 respectively (see text).

the case of terrestrial, relatively sedentary animals like frogs. However, in two other species gut contents appear to have yielded misleading measures of diet variation. For instance, isotopes suggest that *Eleutherodactylus* sp. has a much lower degree of individual specialization than the other species (expected *WIC/TNW* = 0.86). Since there is no *a priori* way of knowing how well gut contents will perform, we do not recommend the use of gut contents alone in studies of individual specialization, unless individuals are repeatedly sampled over time. In the case of 'snapshot' samples, other measures of temporal consistency (e.g. morphology, stable isotopes) should be used as a complementary approach.

It is reasonably easy to understand how gut contents would lead one to overestimate levels of individual specialization ('false specialists'; Warburton et al. 1998). If all individuals had similar preferences (low individual specialization), one may nevertheless see substantial variation among stomachs due to stochastic effects associated with patchy prey distributions, or limited stomach volume so that each consumer holds only a few prey at a time (Bolnick et al., 2002). This appears to be the case in *Eleutherodactylus* sp., since isotopes indicated that there was far less diet variation than we observed in gut contents (*WIC/TNW* = 0.86 and 0.46 respectively). *Eleutherodactylus* sp. is a small-sized frog (mean ± SD SVL = 14.5 ± 2.65 mm; *N* = 124) with small stomach capacity (mean ± SD number of prey items per stomach = 4.0 ± 2.42) that is found both on the ground and on the vegetation (up to 1 m high; A. A. Giaretta, pers. obs.). These two microhabitats may constitute different 'patches' in terms of prey availability, which combined with the low stomach capacity of individuals generated false specialists.

It is more difficult to see why stomach contents would underestimate diet variation as compared to isotope variance, as in *Proceratophrys* sp. We propose three possible explanations for this conflict. First, if prey isotopic signatures vary temporally or spatially, individuals feeding on the same prey taxa but collected in different times or places will show variation in signatures, so that one will observe isotopic variance that is not actually related to diet variation (Matthews & Mazumder 2004, 2005). In our study, we tried to mitigate this problem by testing for seasonal and spatial effects on the consumers' isotopic ratios, but we acknowledge we cannot rule out those effects entirely. Ideally, future studies should strive to assess the seasonal and spatial patterns of variation in the prey isotopic landscape by sampling prey isotopes in the field over the seasons and over space. Bearing those caveats in mind, we did not find any among-site or seasonal differences in the isotopes of *Proceratophrys* sp. (Table S2), indicating that spatial and seasonal variation in prey isotopes seems an unlikely explanation for the apparent conflict between gut contents and isotopes in this species.

Second, the preservation times before the isotopic analysis differed among individuals. If there are consistent shifts in isotopic signatures related to the time of preservation, this could have increased the isotopic variances of our samples. We tested isotopic signatures of our samples against time of preservation and found a positive relationship in *Proceratophrys* sp. ($r^2 = 0.133$; $F_{1,53} = 8.152$; $P = 0.006$; Fig. S2), but not in the other species (all P-values > 0.56; Fig. S2). This significant relationship, albeit weak, may indicate either an effect of preservation time or a temporal trend in the prey isotopic ratios. We see the former as an unlikely explanation for two reasons. First, Kaehler and Pakhomov (2001) and Sweeting et al. (2004) observed that after an initial period of isotopic shifts (four weeks in the former and one day in the latter study) due to formalin or ethanol preservation of animal tissues, isotopic signatures remained stable for the whole duration of experiments (12 weeks in the former and 21 months in the latter). Since all our samples were analyzed after at least 45 months of preservation, we would not expect to see such a trend in the isotopic ratios of our samples due to the effect of preservatives. Second, if preservatives were causing this shift, we would expect to see it in all the four species, because all samples were subject to the same type of preservation. We therefore believe it is more likely that this pattern reflects a temporal trend in one or a few food sources that were more consumed by *Proceratophrys* sp. than the other species (e.g. seeds; Table S1). Either case, future studies would benefit from standardizing the time of preservation of samples so that biases in the isotopic variance due to preservative-induced isotopic shifts will be avoided.

Finally, the very low isotope-derived value of *WIC/TNW* in *Proceratophrys* sp. might be a result of under-sampling prey isotope variation (see below). It is likely that some individuals in our sample fed on some unknown prey with isotope signatures outside the range of what we observed in the most common prey. This is because some individual frogs had isotope signatures outside the range of prey isotopes (Fig. 2), and in *Proceratophrys* sp. the number of isotopic outliers was higher than in the other species (Fig. 2).

The mismatch between frogs' and prey signatures in our samples may have several reasons. First, consumers may show shifts in $\delta^{13}C$ in relation to their food sources due to fractionation (Vander Zanden & Rasmussen 2001). Second, we may have lacked the taxonomic resolution that would have allowed us to measure all the isotopic range of the consumed prey. While we made an effort in trying to avoid lumping ecologically divergent prey types into a single category, our taxonomic resolution (23 prey taxonomic categories, some including many families) almost certainly mixed prey with different signatures. The estimated average values for each category therefore masks a potentially higher variation among member taxa. It is possible that the prey signatures that would encompass all frog signatures in our samples are among these lumped taxa. Third, we may have actually missed some prey taxa in our sample. In principle, this should not be a likely explanation, given our large sample sizes. Moreover, we would not expect those missing prey types to be used frequently enough to strongly influence the frogs' isotopic ratios. This argument also holds for the taxa lumped under the category 'Others', which were not included in the isotope analyses for lack of material. None of these taxa accounted for more than 1% of prey items within any species' diet. Interestingly, however, in the case of *Proceratophrys* sp., all isotopic outliers are small juveniles (all below the 25th percentile of SVL). Differently from the other studied species, *Proceratophrys* sp. reproduces in permanent streams and has a long (many weeks; A. A. Giaretta, unpubl. results) aquatic larval development, whereas *Adenomera* sp. and *Eleutherodactylus* sp. have totally terrestrial development, and *L. fuscus* has a very short (two weeks maximum) aquatic larval phase (Kokubum & Giaretta 2005; A. A. Giaretta, unpubl. results). The feeding habits of the larvae of *Proceratophrys* sp. is unknown, but the diet of tadpoles may well include isotopically depleted sources found in the aquatic environment (Matthews & Mazumder 2005; Paterson *et al.* 2006). Since we only sampled prey consumed in the terrestrial environment, this would explain why the isotopic range in this species was much larger than that of the sampled prey.

Impact of missing prey categories

Our simulation model is based on the assumption that we have a sufficient sample of prey taxa to generate a realistic relationship between *WIC/TNW* and Varδ_i. Underestimating the true variance in prey isotopes could lead to spurious estimates of *WIC/TNW.* To understand why this is the case, consider the *y*-intercept of the simulated curves (Fig. 3). This is the isotopic variance when each individual uses a single prey type (hence *WIC/TNW* = 0), and will be equal to the variance in the empirically determined prey isotopes (weighted by prey frequency in the population diet). Consequently, greater variances in the estimated prey isotopes will generate steeper regression curves. If one underestimates the true prey isotope variance (due to the problems discussed above), the simulated curve will be lower than it should actually be (Fig. 4a). As a result, a given empirical value of Varδ_i will lead to an interpolated *WIC/TNW* that is too low (overestimating individual specialization; Fig. 4a). This observation is of utmost importance for our results, because we used empirically estimated δ^{13}C variances to generate expected values of *WIC/TNW.* If we underestimated the variance in prey isotopes, as suggested by the isotopic outliers in *Proceratophrys* sp., then our regression curves are less steep than they should be, and we might have overestimated the degree of individual specialization. Conversely, overestimating prey variances, for instance by missing isotopically intermediate prey, will lead to an underestimate of individual specialization (higher *WIC/TNW*).

In light of these biases, our interpolation technique is most appropriate when isotope data are available for all prey taxa. This was not possible in this study due to the coarse taxonomic resolution for observed prey and/or our inability to ensure that all prey taxa were accounted for. Since we are unable to determine which prey isotope values we are missing, we took another approach to evaluating the impact of missing prey on our results. We redid our analysis of *Proceratophrys* sp., eliminating the individual frogs whose isotope signatures could not be explained by the observed prey isotopes (see Electronic Supplementary Material 5 for details). Eliminating the 10 isotopic outliers reduced the $\delta^{13}C$ variance from 8.35 to 3.32, but did not effectively change the gut-content estimates of *WIC/TNW* (0.37 vs. 0.38). In contrast, the isotope-derived estimates of *WIC/TNW* increased from 0.16 to 0.52, coming closer in line with the gut content estimates, and the values for two of the other species (Table S4, Fig. S3). This supports our view that the low isotope-derived value of *WIC/TNW* observed in *Proceratophrys* sp. may be a result of insufficient data on prey isotopes. Similar reanalysis increased the estimates of *WIC/TNW* of *Adenomera* sp. from ~0.40 to ~0.55 and had negligible effects on results for the other species (Electronic Supplementary Material 5), which had fewer isotopic outliers.

Impact of the variation in fractionation among individuals

As mentioned earlier, differential assimilation, fractionation and isotopic routing may all cause a mismatch between signatures of food sources and those of consumers. More important, if there is variation among individuals in e.g. fractionation, the population isotopic variance will be higher than would be expected based solely on diet variation (Matthews & Mazumder 2005). As a way of assessing the impact of fractionation on our model, we did simulations incorporating among-individual variance in fractionation (Var_{Δ}). We computed from the literature empirical measures of variation in fractionation among individual fed on the same diet. We used an average $Var_\Delta = 0.73$

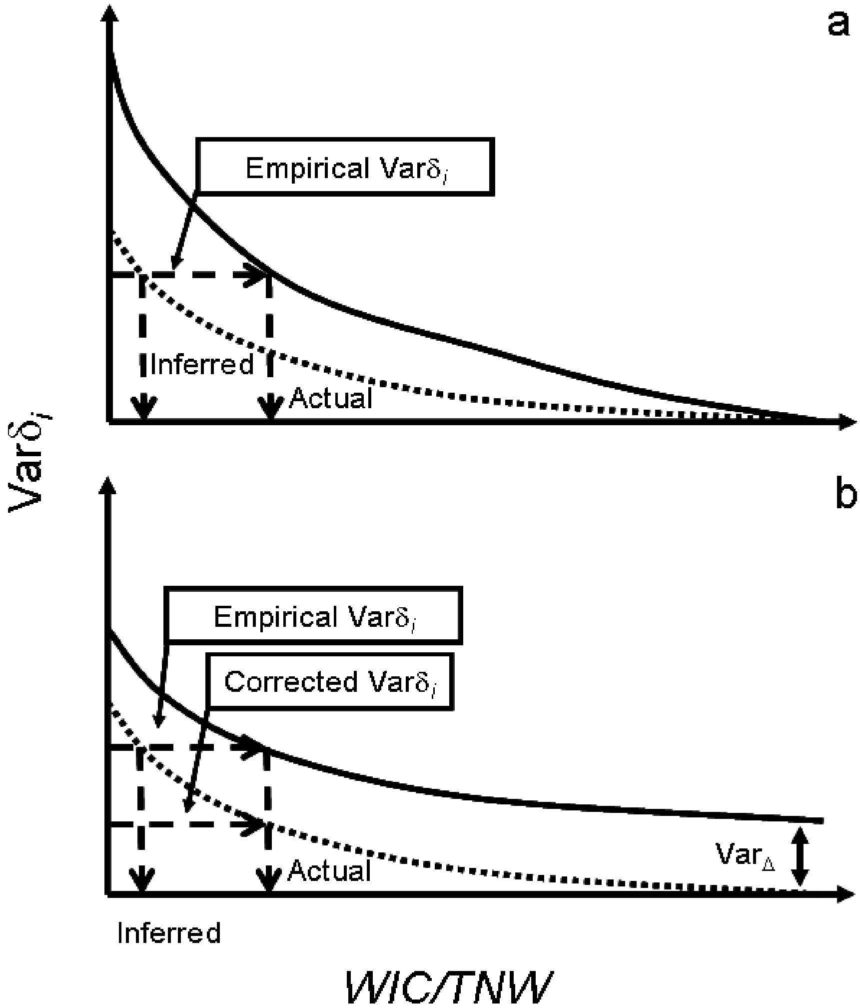

Fig. 4. Illustration of the effect of (**a**) incomplete sampling of prey on our model, and (**b**) variation in fractionation among individuals, based on simulations. **a** The solid curve represents the "true" relationship for a hypothetical prey community, while the dotted curve represents the relationship that is inferred from an incomplete sample of prey that missed isotopically extreme taxa and so underestimates the prey isotope variance. Using this incomplete dataset, one would infer an excessively low value of *WIC/TNW*. **b** Solid curve as in **a**, but now assuming that individuals vary in fractionation, while in the dotted curve no fractionation is assumed. By denying the among-individual variance in fractionation (Var_Δ), one would also underestimate *WIC/TNW*. However, if an empirical estimate of Var_Δ is available, it is possible to correct the estimate of *WIC/TNW* by using a "corrected" $Var\delta_i$, where corrected $Var\delta_i$ = empirical Var $\delta_i - Var_\Delta$.

based on 12 such variances, nine from the gerbil *Meriones unguienlatus* (Tieszen et al. 1983), and one from each of three bird species, the quail *Coturnix japonica*, the chicken *Gallus gallus*, and the gull *Larus delawarensis* (Hobson & Clark 1992). We ran simulations in which a fractionation value drew randomly from a uniform distribution with variance 0.73 (range 0 - 2.96) was added to an individual's isotopic signature. In this simulations, sample size was set at N = 30, population diet was (0.2, 0.2, 0.2, 0.2, 0.2) and prey isotopes were (-31, -30, -29, -28, -27). The incorporation of Var_{Δ} in our model caused an upward shift in the resulting curve, so that even in the absence of diet variation (*WIC/TNW* = 1), there was a baseline isotopic variation (Fig. 4b). Interestingly, the difference between the y-values of this curve and that of a control curve generated with the same set of parameters but no fractionation, corresponds to ~ Var_{Δ} (Fig. 4b). Additional simulations changing the value of Var_{Δ} (0.5 and 1.0) and the prey isotopes range (-34, -32, -30, -28, -26) did not change this pattern. Therefore, if one has an estimate of Var_{Δ} for the studied organism, it is possible to correct its effect on the results of the model by subtracting Var_{Δ} from the empirical $\mathrm{Var}\delta_i$ before interpolating the expected value of *WIC/TNW* (Fig. 4b). Using $\mathrm{Var}_{\Delta} = 0.73$ as a correction for our samples, the expected *WIC/TNW* values increased by 0.05-0.1, indicating less diet variation. The change was not substantial, though, and there is still evidence of diet variation in three of the four species. It is worth mentioning that empirical estimates of Var_{Δ} may vary considerably among different taxonomic groups (e.g. gerbils = 1.01; birds = 0.4; average values). A more realistic estimate in the case of frogs might be quite different from 0.73.

Conclusions

Gut contents may be a useful source of information on individual-level diet variation, especially if coupled with data on stable isotopes. Information on the population $\delta^{13}C$ variance, combined with information on prey isotopes, can be a useful tool to test for the presence of individual specialization (Matthews & Mazumder 2004). The model presented here goes a step further by providing a way to generate, from information on isotopic variances, estimates of standardized indices of individual specialization (Bolnick et al. 2002) that can be compared among different populations or used to evaluate gut-content variation in different species and/or systems. This method requires thorough sampling of the isotope ratios of the prey community, but is otherwise straightforward. Individual specialization is a phenomenon with important ecological and evolutionary implications for populations. In a review of the incidence of individual specialization, Bolnick et al. (2003) make the case that most studies on individual specialization up to now were only able to test the null hypothesis that individuals in a population are all generalists, and that we should be able to actually measure and compare the degrees of individual specialization across different populations. For instance, it is still unclear how widespread this phenomenon is among natural populations, as well as what ecological conditions will favor its evolution and maintenance. Quantifying individual specialization in a comparable manner is a necessary step in any attempt to answer these questions.

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Electronic Supplementary Material 1

Diet composition of four species of Brazilian frogs, and the $\delta^{13}C$ isotope ratios and dry masses of their prey.

Table S1. Diet composition of four species of Brazilian frogs, represented as the number of prey items consumed, and their proportions (in parenthesis) in each species diet. $\delta^{13}C$ signatures and mean dry masses refer to prey categories (numbers in parenthesis indicate number of items analyzed).

Prey categories	$\delta^{13}C$ (‰)	Dry mass (mg)	Species			
			Aden	*Eleu*	*Pro*	*Lep fus*
Collembola	-19.86 (21)	0.02 (19)	5 (0.01)	22 (0.10)	0	0
Orthoptera	-18.64 (6)	8.29 (32)	4 (0.01)	10 (0.05)	16 (0.09)	32 (0.10)
Blattodea	-21.67 (11)	1.58 (20)	22 (0.06)	7 (0.03)	14 (0.08)	18 (0.05)
Isoptera	-14.66 (8)	1.77 (120)	44 (0.12)	7 (0.03)	9 (0.05)	58 (0.18)
Heteroptera (H)[a]	-24.57 (5)	0.87 (10)	5 (0.01)	0	7 (0.04)	2 (0.01)
Heteroptera (P)[b]	-17.44 (6)	1.33 (3)	4 (0.01)	2 (0.01)	3 (0.02)	0
Heteroptera (G)[c]	-22.66 (5)	4.59 (4)	1 (0.00)	1 (0.00)	1 (0.01)	3 (0.01)
“Homoptera”[d]	-16.34 (11)	0.46 (39)	18 (0.05)	23 (0.10)	13 (0.07)	18 (0.05)
Coleoptera (H)[e]	-17.21 (6)	4.43 (25)	1 (0.00)	4 (0.02)	3 (0.02)	14 (0.04)
Coleoptera (P)[f]	-17.22 (18)	1.59 (57)	30 (0.08)	11 (0.05)	10 (0.05)	21 (0.06)
Coleoptera (F)[g]	-19.72 (6)	0.25 (3)	4 (0.01)	0	0	1 (0.00)
Coleoptera (DWC)[h]	-19.39 (6)	0.25 (3)	1 (0.00)	3 (0.01)	0	2 (0.01)
Diptera	-16.19 (5)	0.18 (23)	33 (0.09)	5 (0.02)	6 (0.03)	9 (0.03)
Formicidae	-22.84 (52)	0.35 (202)	94 (0.26)	46 (0.21)	12 (0.06)	59 (0.18)

Hymenoptera Non-Formicidae	-15.10 (4)	1.51 (2)	4 (0.01)	4 (0.02)	2 (0.01)	2 (0.01)
Chilopoda	-13.32 (2)	11.52 (8)	1 (0.00)	5 (0.02)	5 (0.03)	4 (0.01)
Diplopoda	-20.77 (2)	2.49 (4)	6 (0.02)	0	5 (0.03)	0
Araneae	-22.28 (21)	2.04 (82)	37 (0.10)	42 (0.19)	13 (0.07)	25 (0.08)
Opiliones	-13.51 (1)	0.40 (10)	4 (0.01)	4 (0.02)	6 (0.03)	1 (0.00)
Isopoda	-13.34 (1)	1.47 (10)	2 (0.01)	0	10 (0.05)	1 (0.00)
Gastropoda	-18.66 (4)	0.18 (10)	8 (0.02)	6 (0.03)	2 (0.01)	2 (0.01)
Oligochaeta	-16.16 (3)	1.63 (2)	0	0	1 (0.01)	2 (0.01)
Seeds	-17.18 (12)	0.83 (12)	8 (0.02)	0	11 (0.06)	8 (0.02)
Others[i]	–	–	22 (0.06)	20 (0.09)	36 (0.19)	46 (0.14)
Total	–	–	358 (1.00)	222 (1.00)	185 (1.00)	328 (1.00)

Aden: *Adenomera* sp. (*N* = 58); *Eleu*: *Eleutherodactylus* sp. (*N* = 56); *Pro*: *Proceratophrys* sp. (*N* = 49); *Lep fus*: *Leptodactylus fuscus* (*N* = 57). H: herbivores; P: predators; G: granivores; F: fungivores; DWC: dead-wood consumers; the category "Others" was not included in the isotope analyses due to insufficient material. a, Berytidae, Coreidae, Lygaeidae, Miridae, Pentatomidae, Pyrrhocoridae, Rhopalidae, and Tingidae; b, Gelastocoridae, Ploiariidae, Reduvidae, and Veliidae; c, Cydnidae; d, "Homoptera" = Sternorrhyncha + Auchenorrhyncha; e, Chrysomelidae, and Scarabaeidae; f, Cantharidae, Carabidae, Coccinelidae, Dytiscidae (larvae), Elateridae, Lampyridae, Pselaphidae, and Staphylinidae; g, Endomychidae, Limulodidae, and Nitidulidae; h, Bostrichidae, Cerambycidae, and Scolytidae; i, Arthropoda NI (NI: non-identified), Insecta NI (larvae and adults), Odonata (naiads), Dermaptera, Psocoptera, Neuroptera, Hemiptera NI, Coleoptera NI (larvae and adults), Tenebrionidae, Lepidoptera (larvae and adults), Scorpiones, Pseudoscorpiones, Acari, Crustacea NI, Annelida NI, Anura (Hylidae).

ELECTRONIC SUPPLEMENTARY MATERIAL 2

Effect of sex, age class, site of collection, and seasonality on the diets and $\delta^{13}C$ isotope ratios of four Brazilian frogs.

Table S2. Tests of the effect of sex, age class (juveniles and adults), site of collection (five sites), and season (wet and dry) on the diets and $\delta^{13}C$ isotopic signatures of four Brazilian frogs. We did multi-way MANOVA's and ANOVA's on diet and isotope data respectively. In the MANOVA's, the PCA scores of the major axes (axes that explain > 5% of the variation) of diet variation were used as dependent variables. Significant *P*-values are in bold face.

Species	Diet		$\delta^{13}C$		
	MANOVA		ANOVA		
	Wilks' Λ	*P*	*F*	df	*P*
Adenomera sp.					
sex	0.825	0.340	0.006	1,46	0.939
site	0.568	0.301	5.433	3,46	**0.003**
season	0.926	0.889	0.471	1,46	0.496
Eleutherodactylus sp.					
sex	0.696	0.169	0.000	1,42	0.998
site	0.297	0.165	1.144	4,42	0.349
season	0.732	0.282	0.000	1,42	0.989
Proceratophrys sp.					
sex	0.873	0.899	2.359	1,38	0.133
age	0.636	0.095	0.001	1,38	0.974
site	0.211	**0.002**	0.534	1,38	0.469
season	0.853	0.840	0.952	1,53	0.334
L. fuscus					
sex	0.848	0.721	0.034	1,35	0.855
age	0.792	0.439	0.157	1,35	0.694
site	0.337	0.177	8.248	4,35	**< 0.001**
season	0.775	0.363	0.065	1,35	0.800

Diet and isotope sample sizes were: *Adenomera* sp. (*N* = 57; *N* = 59); *Eleutherodactylus* sp. (*N* = 55; *N* = 59); *Proceratophrys* sp. (*N* = 48; *N* = 53); and *Leptodactylus fuscus* (*N* = 56; *N* = 59).

ELECTRONIC SUPPLEMENTARY MATERIAL 3

Isotope/gut content comparison for a second index of individual specialization.

Bolnick et al. (2002) present three indices of individual specialization that can be used with categorical data (e.g. prey taxa). They discuss several of the indices properties, but do not define one best index. When writing the code, we decided to include also the *IS* index of individual specialization, first as a way to double check the results of *WIC/TNW*, second to give the users a second option of index. The *IS* index is based on Schoener's (1968) proportional similarity index, *PS*, which is a measure of the degree of diet overlap originally designed for inter-specific comparisons, but can be used to measure the overlap between individuals and the population as a way to infer individual specialization. Since individual specialists are defined as individuals that use a small subset of the population's range of resources, we can quantify the level of individual specialization by comparing an individual's resource use distribution to that of the population as a whole. If individuals use the same range of resources as the population as a whole, their diet distributions will be broadly overlapping ($PS \sim 1.0$). In contrast, an individual that uses only a single resource type j will have a $PS = p_j$. Hence, lower overlap with the population diet indicates stronger individual specialization. We summarized the population-wide degree of individual specialization as the mean of *PS* values (*IS*). The closer *IS* is to 0, the higher the degree of individual specialization in the population, and the closer it is to 1 the more generalized are the individuals.

The results for *IS* were qualitatively similar to those for *WIC/TNW*. There was evidence of individual specialization based on gut-content data (Table S3). The observed values of *IS* were significant for all species except *Eleutherodactylus* sp., in which it was marginally significant ($P = 0.093$). As in the case of *WIC/TNW*, simulations confirmed that there is also a relationship between *IS*, and the variance in stable isotope ratios among individuals $\mathrm{Var}\delta_i$ (Fig. S1). We used the regression equations (Fig. S1) to calculate the expected values of *IS* (Table S3; Fig. S1). Consistent with the results of *WIC/TNW*, gut contents underestimated diet variation in *Proceratophrys* sp. (Fig. S1), overestimated it in *L. fuscus* (Fig. S1), and greatly overestimated it in *Eleutherodactylus* sp. (Fig. S1).

Table S3. Measures of intra-population diet variation (*IS*) in four species of Brazilian frogs. IS_{obs}: index based on gut-content data; IS_{exp}: expected value of the same index based on model. $\mathrm{Var}\,\delta^{13}\mathrm{C}$: empirically estimated isotopic variances of frog samples. See text for details on the model. Numbers in parenthesis are sample sizes. *L. fuscus*: *Leptodactylus fuscus*. $^{*}P = 0.01$; $^{***}P < 0.001$ (Monte Carlo bootstraps; 1000 simulations). ss1 and ss2: subsamples 1 and 2 respectively (see text for details).

Species	IS_{obs}	$\mathrm{Var}\delta^{13}\mathrm{C}$	IS_{exp}
Adenomera sp.			
ss1 (39)	0.3559^{**}	5.38	0.2874
ss2 (35)	0.3737^{**}	4.90	0.4983
Eleutherodactylus sp. (56)	0.3223^{NS}	1.38	0.7305
Proceratophrys sp. (49)	0.1829^{***}	8.35	0.0330
L. fuscus			
ss1 (38)	0.2838^{**}	2.87	0.3643
ss2 (29)	0.2901^{***}	2.01	0.5348

Sample sizes in parenthesis. NS: non-significant; $^{**}P < 0.01$; $^{***}P < 0.001$ (Monte Carlo bootstraps; 1000 simulations).

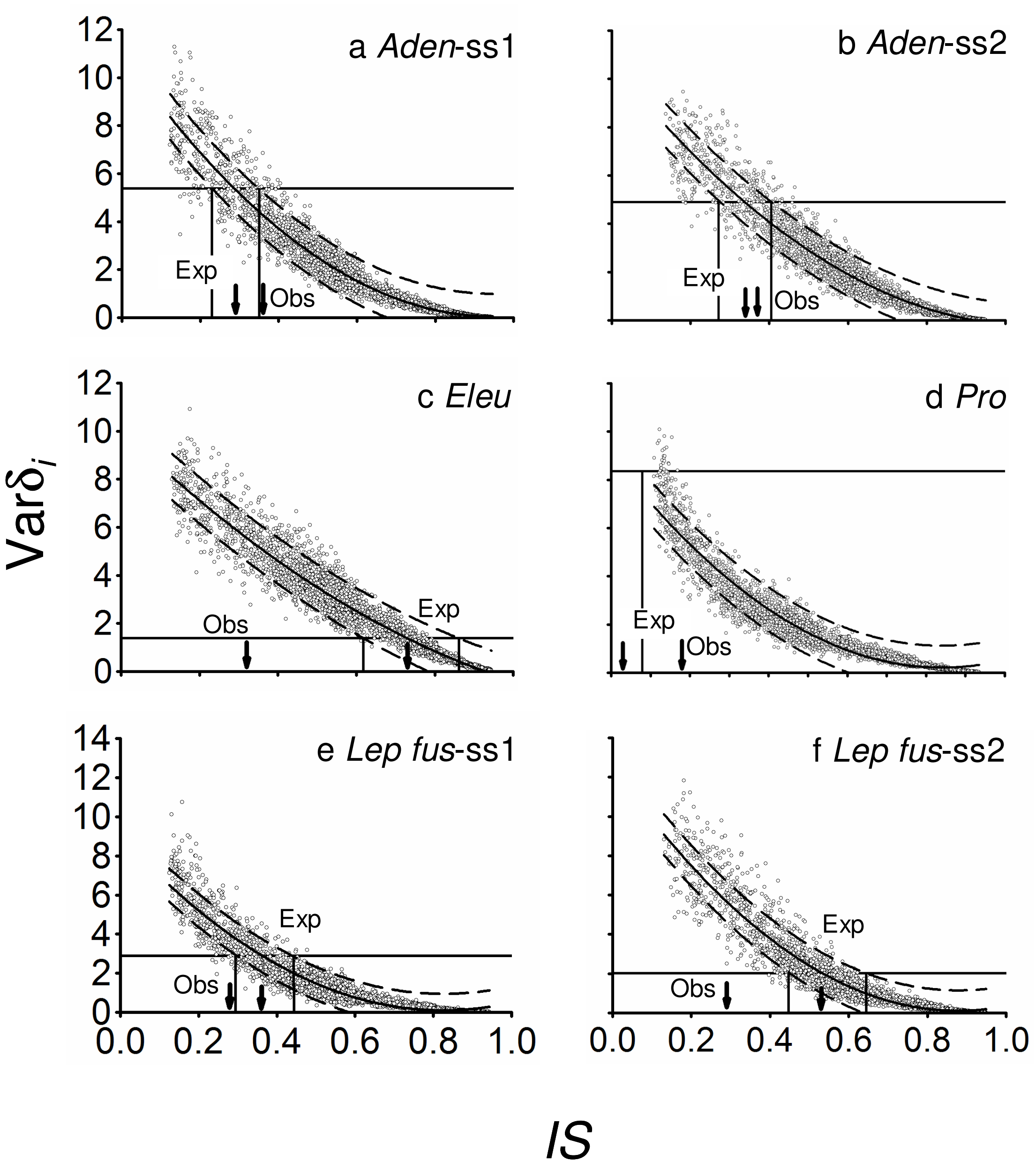

Fig. S1. Same as Fig. 3 in the main text, but using the *IS* measure of individual specialization: **a** *Adenomera* sp.-ss1 (N = 39); **b** *Adenomera* sp.-ss2 (N = 35); **c** *Eleutherodactylus* sp. (N = 56); **d** *Proceratophrys* sp. (N = 55); **e** *Leptodactylus fuscus*-ss1 (N = 38); **f** *Leptodactylus fuscus*-ss2 (N = 29); ss1 and ss2: subsamples 1 and 2 respectively (see main text). Exp: expected; Obs: observed.

ELECTRONIC SUPPLEMENTARY MATERIAL 4

Effect of time of preservation in $\delta^{13}C$ signatures.

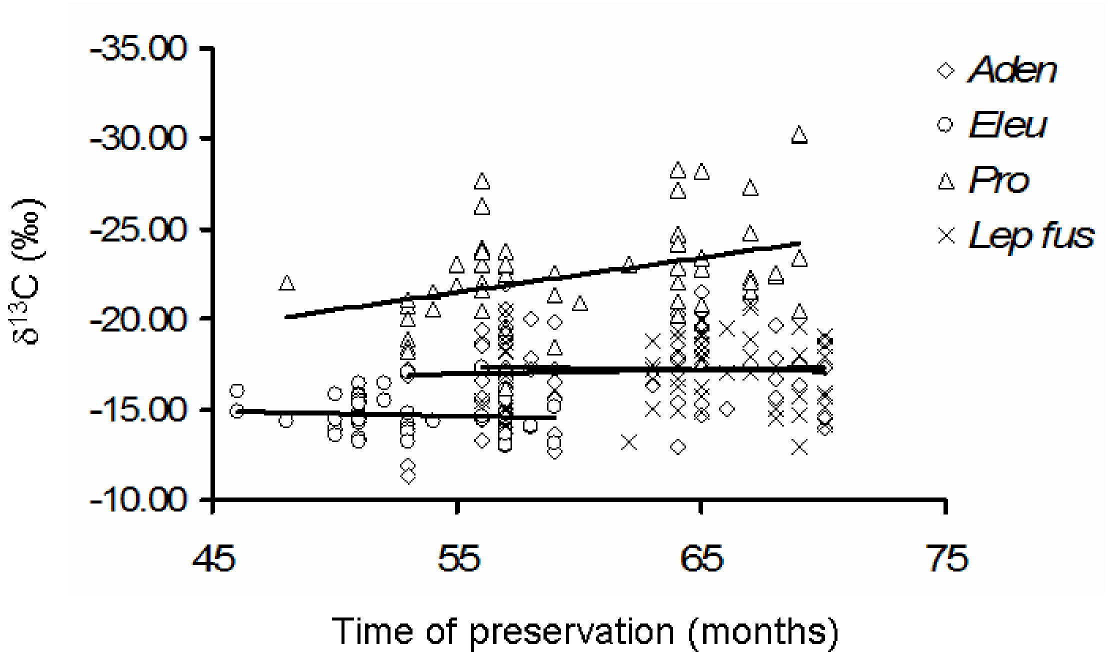

Fig. S2. $\delta^{13}C$ signatures against time of preservation in four populations of Brazilian frogs. *Aden*: *Adenomera* sp. (*N* = 60); *Eleu*: *Eleutherodactylus* sp. (*N* = 60); *Pro*: *Proceratophrys* sp. (*N* = 55); *Lep fus*: *Leptodactylus fuscus* (*N* = 60).

ELECTRONIC SUPPLEMENTARY MATERIAL 5

Results of simulations after culling isotope outliers.

Table S4. Inferred values of *WIC/TNW* after culling individuals whose isotopic signatures were outside the estimated isotopic prey range for each population.

Species	WIC/TNW_{obs}	Var δ^{13}C	WIC/TNW_{exp}
Adenomera sp.			
ss1 (34)	0.4970***	3.18	0.6251
ss2 (32)	0.4334***	3.85	0.5044
Eleutherodactylus sp. (49)	0.4641***	1.21	0.8972
Proceratophrys sp. (40)	0.3759***	3.32	0.5237
L. fuscus			
ss1 (36)	0.4988***	2.46	0.5320

Sample sizes in parenthesis. $^{***}P < 0.001$ (Monte Carlo bootstraps; 1000 simulations).

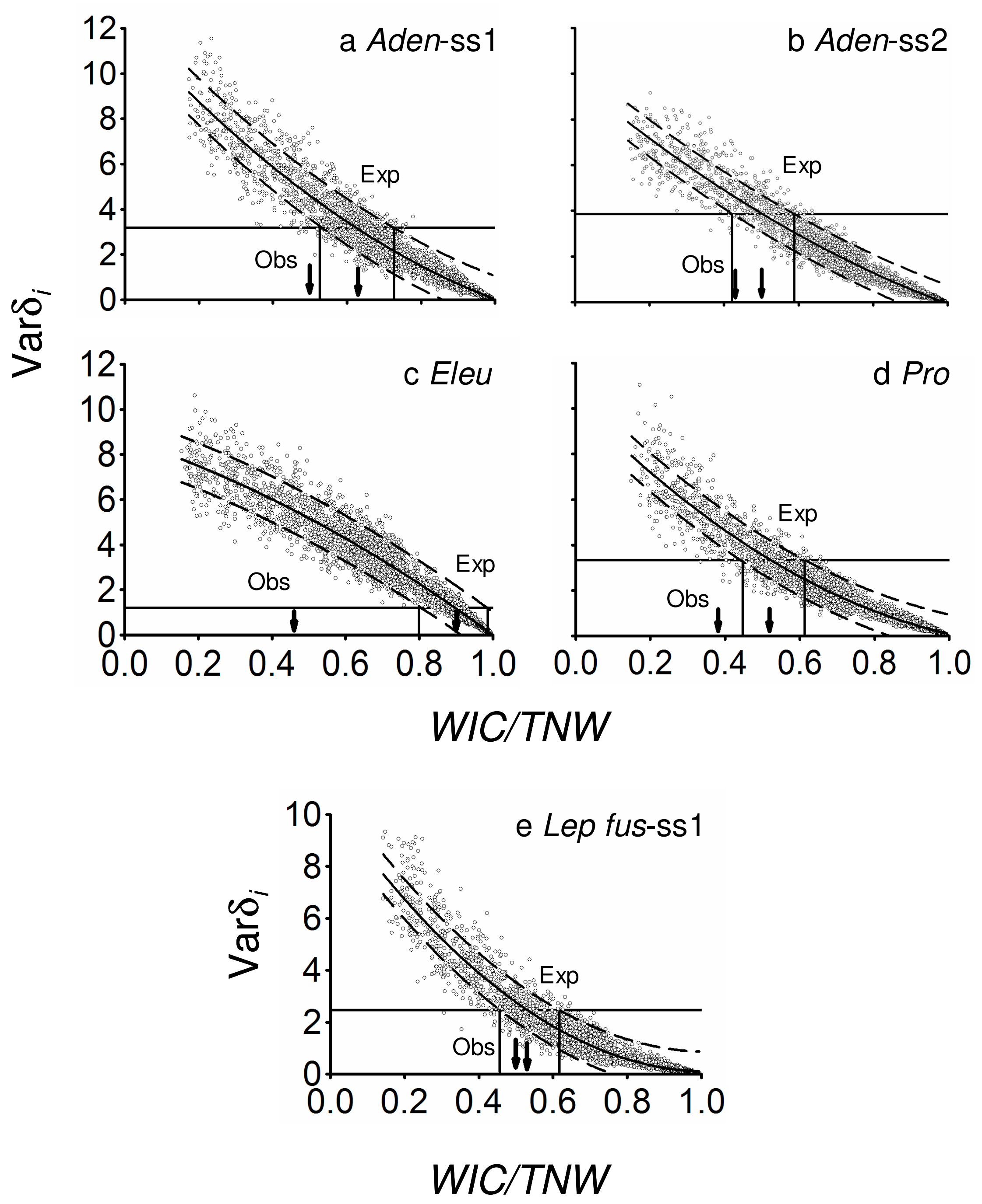

Fig. S3. Same as Fig. 3 in the main text, but individuals with isotopic signatures out of the prey range were culled from the analyses. **a** *Adenomera* sp.-ss1 (*N* = 34); **b** *Adenomera* sp.-ss2 (*N* = 32); **c** *Eleutherodactylus* sp. (*N* = 49); **d** *Proceratophrys* sp. (*N* = 40); **e** *Leptodactylus fuscus*-ss1 (*N* = 36). Exp: expected; Obs: observed.

Capítulo 3

Individual specialization in the hunting-wasp *Trypoxylon* (*Trypargilum*) *albonigrum* (Hymenoptera, Crabronidae)
Araújo, M.S. & M.O. Gonzaga
Behavioral Ecology & Sociobiology (no prelo)

Individual Specialization in the Hunting-Wasp *Trypoxylon* (*Trypargilum*) *albonigrum* (Hymenoptera, Crabronidae)

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ABSTRACT

Individual-level variation in resource use occurs in a broad array of vertebrate and invertebrate taxa and may have important ecological and evolutionary implications. In this study, we measured the degree of individual-level variation in prey preference of the hunting-wasp *Trypoxylon albonigrum*, which inhabits the Atlantic Forest in southeastern Brazil. This wasp captures several orb-weaving spider genera to provision nests. Individuals consistently specialized on a narrow subset of the prey taxa consumed by the population, indicating the existence of significant individual-level variation in prey preferences. The population niche was broader in the wet season in terms of both prey size and taxa. In the case of prey size, the population niche expansion was achieved via increased individual niche breadths, whereas in the case of prey taxa individual niches remained relatively constrained and the population niche expanded via increased interindividual variation. The observed pattern suggests the possibility of functional trade-offs associated with the taxon of the consumed prey. The nature of the trade-offs remains unknown, but they are likely related to learning in searching and/or handling prey. We hypothesize that by specializing on specific prey taxa individuals increase foraging efficiency, reducing foraging time and ultimately increasing reproductive success.

Key-words: Apoidea; learning trade-offs; niche variation; intra-population variation

INTRODUCTION

Many natural populations are composed of ecologically heterogeneous individuals that use different subsets of the available resources (Van Valen 1965; Heinrich 1979; Lewis 1986; West 1986; Price 1987; Werner & Sherry 1987; West 1988). Individuals within a population may vary in resource use because they inhabit different microhabitats (Durell 2000), or due to sex and age-related morphological or behavioral differences (Polis 1984; Slatkin 1984). However, individuals can also exhibit niche variation within sex or age-class, and within a single site or time. For example, in the finches *Pinaroloxias inornata* (Passeriformes, Emberizidae) of Cocos Island, Costa Rica, individuals feed as specialists, while the population as a whole is extremely generalized in its foraging habits and exploits a broad range of food types (Werner & Sherry 1987). This individual-level variation is called “individual specialization” and may have several ecological and evolutionary implications (Bolnick et al. 2003).

Individual specialization is generally related to constraints on an individual’s ability to efficiently exploit a wide variety of resources. Constraints generally arise from functional trade-offs, in which consumers efficiently exploiting one type of resource are inefficient using another type of resource (Bolnick et al. 2003). If resource use involves learning, neurological limitations may prevent individuals from using a resource once they have learned how to exploit a different resource (Werner *et al.* 1981; Lewis 1986; Werner & Sherry 1987; Bernays & Funk 1999). For instance, in the cabbage butterfly

Pieris rapae (Lepidoptera, Pieridae), the efficiency of individuals in extracting nectar from flowers increases with time following a learning curve, and individuals show consistency through time on the type of flower exploited (Lewis 1986).

The variety of resources used by a population may vary according to changes in the abundance of resources in the environment. For example, temporally abundant resources may be included in the population diet causing the population niche to expand (Schoener 1986; Robinson & Wilson 1998). This niche expansion in turn can be achieved in two ways. Every individual in the population may use a broader array of resources, or there can be greater interindividual variation (Bolnick et al. 2003). The latter scenario is more in line with the presence of trade-offs, as individual niches remain constrained while the population niche expands, so that individuals use only a subset of the resources used by the population as a whole, causing individual specialization. This niche expansion via increased interindividual variation has been observed in a natural population of the perch *Perca fluviatilis* (Perciformes, Percidae) from central Sweden (Svanbäck & Persson 2004) and has been experimentally demonstrated in sticklebacks (Svanbäck & Bolnick 2007) and *Drosophila melanogaster* (Bolnick 2001).

Individual specialization in resource use has been demonstrated in a wide array of taxa (Bolnick et al. 2003), and there is some evidence that it may also occur in the hunting-wasp genera *Trypoxylon* (Crabronidae) (Coville & Coville 1980; Coville 1987) and *Chalybion* (Sphecidae) (Muma and Jeffers 1945). Coville and Coville (1980), for example, collected nests of *T. tenoctitlan* in which one female deposited practically only Araneidae prey, while another female provisioned its nest mainly with jumping spiders (Salticidae). According to Coville (1987) the individual-level differences in the prey content of nests may result from females hunting in different areas, exploiting aggregations of spiders, or becoming conditioned to certain types of spiders or hunting behavior. In addition to *Trypoxylon*, three other genera in the family Crabronidae and two in the related family Sphecidae hunt spiders to provide food for their larvae (Coville 1987). These wasps capture a wide range of spider taxa, including orb weaving spiders (e.g. Araneidae, Tetragnathidae, Nephilidae), araneiod sheet web weavers (e.g. Theridiidae), and even several taxa that usually do not use webs to capture prey (e.g. Lycosidae, Salticidae, Oxyopidae, Clubionidae) (Muma & Jeffers 1945; Coville & Coville 1980; Camillo & Brescovit 1999; Blackledge *et al.* 2003). After attacking and immobilizing their prey, the wasps transport the paralyzed spiders to nests constructed inside natural cavities, excavated in the ground or built using mud. An interesting feature of these nests is that they usually contain several chambers (also called ‘cells’), each holding one larva, that are provisioned with several spiders each (Coville 1987, O’Neill 2001). As a result, nests contain a natural register of many foraging events of individual wasps, providing an excellent opportunity to investigate individual consistency in resource use.

In the present study, we investigated the phenomenon of individual specialization in a population of the hunting wasp *Trypoxylon* (*Trypargilum*) *albonigrum*. This wasp builds mud cylindrical nests containing several cells (Fig. 1a) that are provisioned with a large number of spiders of at least six genera of orb-weaving spiders (Araneidae), spanning a variety of sizes, web architectures (presence/absence of retreats and free-sectors, mesh density), and defensive tactics (Gonzaga & Vasconcellos-Neto 2005). For example, in one of the consumed genera, *Parawixia*, spiders remain in the center of the web (Fig. 1b-d) and usually flee to the vegetation when disturbed, whereas the members of another consumed genus, *Eustala*, remain most of the day in a cryptic position close to the vegetation (Fig. 1e-g). Therefore, these different spiders may potentially require different hunting skills from the wasps, imposing learning trade-offs. Thus, we hypothesize that individual wasps show high consistency in prey choice. Additionally, the prey composition of nests changes seasonally in the study site (Gonzaga & Vasconcellos-Neto 2005). Provided that there is individual specialization and the population niche breadth varies seasonally, we have the opportunity to test the hypothesis that the degree of individual specialization increases when the population niche becomes

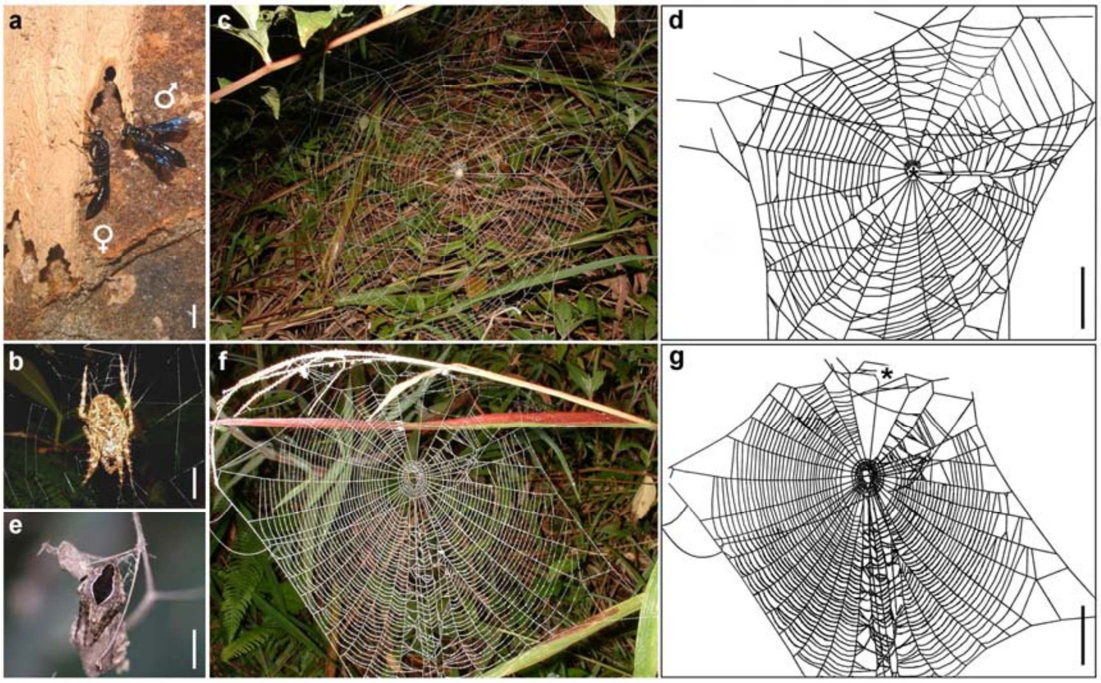

Fig. 1. **a** Female (left) of *Trypoxylon albonigrum* building a mud nest. The individual on the right is the male. **b** Female of *Parawixia audax* **c-d** Web of *P. audax* showing the position occupied by the spider during the day, in the center of the orb (*) **e** Female of *Eustala* sp. **f-g** Web of *Eustala* sp. showing the peripheral position occupied (*) and the free sector in the web. The spider remains in the vegetation, holding a thread connected to the hub of the orb. Scales: **a**, **b**, **e**: 0.5 cm; **c**, **d**, **f**, **g**: 5 cm.

broader as a consequence of individual niches remaining constrained, which would be suggestive of functional trade-offs. Specifically, we (i) quantified the degree of individual specialization in prey size and taxon in *T. albonigrum*, and (ii) tested the hypothesis that broader niches correspond with higher degrees of individual specialization.

METHODS

Study site

We carried out this study at Parque Estadual Intervales, an Atlantic forest reserve of about 49,000 ha located in the Paranapiacaba mountain range, close to Ribeirão Grande, state of São Paulo, Brazil. This reserve is surrounded by three other preservation units, accounting for more than 120,000 ha of continuous old secondary growth and primary evergreen forest. Climate is characterized by a hot/wet season (hereafter 'wet season') from September to March (monthly rainfall varying from 112.5 to 265.5 mm and average temperature between 14.6 to 20.4ºC) and a relatively cold/dry season (hereafter 'dry season') from April to August (63.2-99.2 mm of rainfall and average temperature varying from 14.2 to 16.7 ºC – data collected from 1992-1997 and 2002-2003 in a meteorological station located 10 km from the study site).

Data collection

Nests were sampled in the area known as "Carmo" (24^{o}18'S, 48^{o}24'W), once a month, from December 2001 to November 2002. Nests were found attached to the walls of a house (a research station), either alone or forming sets containing 2 to 6 adjacent nests (Fig 1a). Different sets were considered as belonging to different females. All the adjacent nests in a set were assumed to belong to the same female, but only the last pipe of the set, which was usually being filled with spiders at the moment of sampling, was sampled. We took this procedure because cells in the earlier constructed pipes of a given set usually had well developed larvae that had already partially or entirely consumed the provisions. Since nests were in different stages of conclusion when sampled, the number of cells and, as a consequence, of spiders varied considerably among individual wasps (Fig. 2a).

The body lengths of all intact spiders were measured to the nearest 0.01mm using a dissecting microscope with an ocular micrometer. These length measures were then used to calculate estimates of dry weights based on a regression equation relating dry weight to length for all the spider genera found in this study (Gonzaga & Vasconcellos-Neto 2005). Voucher specimens of *T. albonigrum* were deposited in the Museu de Zoologia da Universidade de São Paulo (curator C.R.F. Brandão) and the spiders were deposited in the collection of the Instituto Butantan (curator A.D. Brescovit), São Paulo, Brazil.

Data analyses

Although we were able to identify most spiders to species level, we lumped species into genera and defined the latter as the prey taxa in the analyses. We did so because all the analyzed species within a genus share the same features (e.g. size, web architecture, defensive tactics) that are probably important cues for the wasps when foraging or may affect their foraging success. Therefore, we think that in this system the spider genera reflect better the different types of resources available to the wasps.

Individual specialization

Prey size.—In order to measure individual specialization in prey size, we used Roughgarden's (1974) measure of individual specialization for continuous data, *WIC/TNW*. The total variance of resources corresponds to the total niche width of the population (*TNW*) and can be divided into two components so that *TNW* = *WIC* + *BIC*.

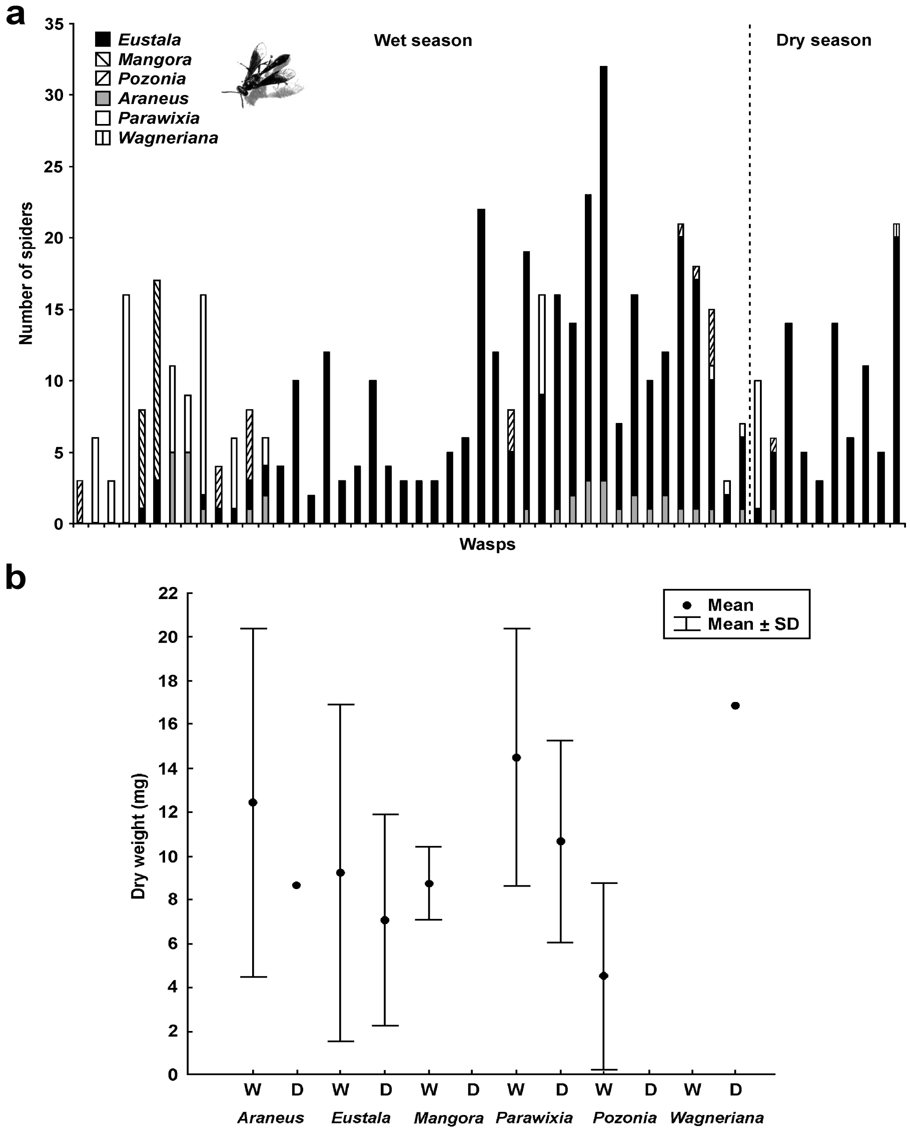

Fig. 2. **a** Number of specimens belonging different spider genera captured by individuals of the hunting-wasp *Trypoxylon albonigrum* during the wet and dry seasons. Each bar represents the content of one nest belonging to one individual wasp **b** Mean and standard deviations (SD) of the dry weights of the spiders captured in each season.

If the niche is measured in terms of prey size, the within-individual component (*WIC*) is the average variance of prey sizes used by individuals and measures the average individual niche width. Variation among individuals is measured by the between-individual component (*BIC*), which is the variance among individuals' mean prey sizes. Traditionally, the degree of diet variation is described by calculating the proportion of total niche variation ascribed to individual niche widths (*WIC/TNW*). The higher the value of *WIC* relative to *TNW*, the less variable individuals are, and vice-versa. Therefore, *WIC/TNW* varies from 0 (maximum individual specialization) to 1 (no individual specialization). It would be more intuitive to use *BIC/TNW* as a measure of interindividual variance, but we stick to Roughgarden's (1974) *WIC/TNW* to follow historical precedence.

Prey taxa.—In the case of prey taxa, we used the adaptation of Schoener's (1968) proportional similarity index, *PS*, to measure individual specialization, which measures the overlap between an individual *i*'s diet and the population diet (Bolnick et al. 2002):

$$PS_i = 1 - 0.5\sum_j \left| p_{ij} - q_j \right|$$

in which PS_i is the overlap between individual *i*'s niche and the population niche, p_{ij} represents the proportion of prey category *j* in individual *i*'s diet and q_j is the proportion of the *j*th resource category in the population's niche and is calculated as

$$q_j = \frac{\sum_i n_{ij}}{\sum_i \sum_j n_{ij}}$$

where n_{ij} represents the number of items in individual *i*'s diet that falls into category *j*. For an individual *i* that specializes on a single prey category *j*, its PS_i will take on q_j, whereas for individuals that consume prey in direct proportion to the population as a whole PS_i will equal 1. 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). An interesting feature of PS_i is that it generates measures of individual specialization for each individual in the population, which allowed us to compare the degree of individual specialization between wet and dry seasons by performing a Mann-Whitney *U* test on PS_i values. The calculation of all indices was performed in IndSpec1, a program to calculate indices of individual specialization (Bolnick et al. 2002). We also used IndSpec1 to calculate the significance of the *WIC/TNW* and the *IS* measures of individual specialization. IndSpec1 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. We used 10,000 replicates in Monte Carlo bootstrap simulations to obtain *P*-values for these indices. The Mann-Whitney *U* test was performed in SYSTAT11.

Population and individual niche breadth

Prey size.—Since the population niche breadth in prey size corresponds to the variance in the size of prey consumed, we compared the niche breadth in prey size between seasons with an *F*-test on the variances of the ln-tranformed dry weights. When sample sizes allowed, we compared the variance in the ln-transformed dry weights between seasons within each prey taxon.

Prey taxa.—The niche breadth of the prey taxa consumed was measured with Levins' *D* (Levins 1968):

$$D = \frac{1}{\sum_j q_j^2}$$

where q_j is the same as above. In the case of individual niches, q_j represents the proportion of the *j*th resource category in an individual's diet and is calculated as

$$q_j = \frac{n_j}{\sum_j n_j}$$

In order to compare the population niche breadth between seasons one needs to calculate sampling error estimates for the niche-breadth measures. We used 10,000 non-parametric bootstrap resamplings to calculate 95% confidence limits, i.e. 2.5 and 97.5 percentiles, around the measures of population niche breadth (Efron & Tibshirani 1993; Davidson & Hinkley 2003). If the confidence limits overlap, there is no significant difference between niche breadths of the dry and wet seasons; niche breadths are significantly different otherwise. We developed a computer program that draws individual niches from an empirical sample of size *N*, with replacement, to build simulated populations of the same size, after which *D* is calculated. The program was written in C language and is available from the authors upon request. Additionally, we compared the individual niche breadths in the dry and wet seasons with a Mann-Whitney *U* test on the individual *D* values. The Mann-Whitney *U* test and the *F*-test were performed in SYSTAT11.

RESULTS

Individual specialization

The average ± standard deviation number of spiders per examined nest was 10.1 ± 6.60 (range 2 – 32; $N = 54$), and individuals showed great consistency in their foraging preferences (Fig. 2a).

Prey size

We found significant individual specialization in prey size in the dry season (*WIC/TNW* = 0.6404; *P* = 0.0118; *N* = 10), but not in the wet season (*WIC/TNW* = 0.8798; *P* = 0.6524; *N* = 44). This was a reflection of the great increase in the within-individual component of the total niche width in the wet season (*WIC* = 1,884.5) as compared to the dry season (*WIC* = 17.8).

Prey taxa

We found evidence of significant individual specialization in prey taxa in the dry (*IS* = 0.8097; *P* = 0.0062; *N* = 10) and wet season (*IS* = 0.5742; *P* < 0.0001; *N* = 44), and the degree of individual specialization was significantly higher (lower *IS* value) in the wet season (Mann-Whitney *U* = 464; *P* < 0.001; Fig. 3a).

Population and individual niche breadths

Prey size

In the wet season, the population used a far broader range of prey sizes (*TNW* = 2,142.0) than in the dry season (*TNW* = 27.73), a result confirmed by the comparison between prey size variances ($F_{93,453}$ = 0.520; *P* < 0.001). Within genera, prey size variance was larger in the wet than in the dry season in *Eustala* and *Parawixia* (Fig. 2b), although the difference was only significant in the former (*Eustala*: $F_{82,339}$ = 1.680; *P* = 0.0053; *Parawixia*: $F_{65,8}$ = 1.122; *P* = 0.72). In the other genera, sample sizes prevented us from comparing the variances statistically.

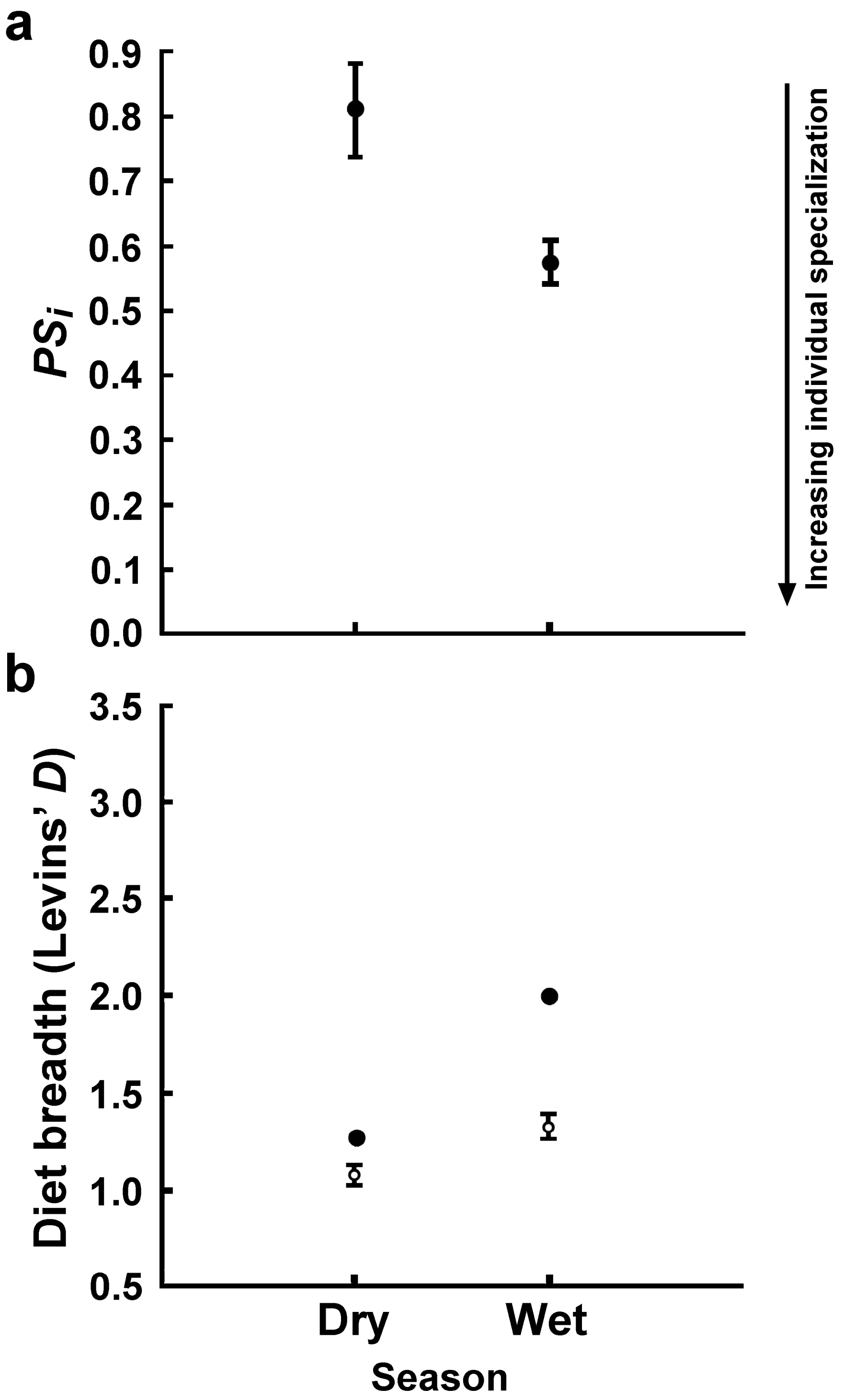

Fig. 3. **a** PS_i measure of individual specialization in the hunting-wasp *Trypoxylon albonigrum* in the dry and wet seasons (mean and standard errors) **b** Population diet breadth (●) and mean (± standard errors) individual diet breadth (○) measured with Levins' *D*. Lower PS_i values indicate stronger individual specialization.

Prey taxa

In the dry season, *T. albonigrum* relied almost entirely on spiders of the genus *Eustala* to provide food for their larvae, whereas in the wet season other spider genera were also highly consumed by some individuals (Fig. 2a). As a consequence, the population niche was broader in the wet (empirical $D = 1.9789$) than in the dry season (empirical $D = 1.2674$; Fig. 3b). There was, however, overlap between the confidence intervals around the D measures of the dry (1.0185 – 1.8674) and the wet season (1.5664 – 2.6497), indicating no significant seasonal difference in niche breadth. The individual niche breadths were not significantly different between seasons either (Mann-Whitney $U = 143$; $P = 0.069$; $N = 54$; Fig. 3b). It is worth mentioning that, although we did not find any significant seasonal differences in niche breadth, the increase in the population niche breadth was disproportionately higher than the increase in the individual niche breadhts (Fig. 3b), indicating that individual niches remain relatively constrained when the population niche expands.

DISCUSSION

There was significant individual specialization in prey size in *T. albonigrum* in the dry season and significant individual specialization in prey taxon in both seasons, indicating that individuals consistently use only part of the resources used by the population. Moreover, individual niches remained relatively constrained in terms of prey taxa as the population niche expanded in the wet season, causing a higher degree of individual specialization. On the other hand, individuals expanded their range of prey sizes in the wet season, as indicated by the higher *WIC* values in this season. In the following paragraphs we discuss (i) the possible mechanism underlying individual specialization, (ii) the temporal consistency of specialization, and (iii) the ecological implications of this individual-level variation in resource use.

Mechanism of individual specialization

An important task in the study of individual specialization is to identify its underlying mechanisms, which is in general associated with the presence of functional trade-offs, either morphological, behavioral or physiological (Bolnick et al. 2003). An increase in foraging efficiency associated with learning has been shown in a wide range of animals, from butterflies, bees, and aphids among insects (Heinrich 1979; Lewis 1986; Bernays & Funk 1999), to fish and birds (Werner & Hall 1974; Werner *et al.* 1981; Werner & Sherry 1987) among vertebrates. In all these cases, individuals tended to become specialists after some experience with a given resource.

In the case of *T. albonigrum*, individual specialization is possibly a result of learning trade-offs associated with prey searching and/or handling times. This wasp preys on spiders showing a wide range of defensive tactics, such as (i) to remain in retreats composed of curled leaves and silk (e.g. *Araneus*), (ii) to move towards the web periphery or the vegetation when disturbed (e.g. *Parawixia*), and (iii) to remain constantly in the vegetation in a cryptic position (e.g. *Eustala*). These differences in the spiders' rest positions and reactions, in turn, may require different skills from wasps to locate and subdue their prey. For example, when hunting the spider *Larinioides cornutus* (Araneidae), the wasp *Sceliphron caementarium* chases it out of its retreat (Eberhard 1970), but when hunting *Argiope aurantia* or *A. trifasciata* (Araneidae), it bumps into the web vigorously, dropping the spider from it, and then pursues the spider by crawling around the vegetation in gradually enlarging circular patterns (Blackledge & Pickett 2000). Additionally, *Trypoxylon politum* (Rau 1944) and *Trypoxylon* sp. (Blackledge & Pickett 2000) may use aggressive mimicry, plucking the web threads to attract resident spiders, and different spider species may require distinct mimicry signals (Jackson & Wilcox 1993). It is clear from these examples that not only different spiders require different hunting techniques, but also these techniques may often be very elaborate.

Now, if learning is an important step in hunting efficiently in wasps and there are learning trade-offs associated with different hunting techniques, we would expect individual wasps to specialize on specific resource types. In support of this idea, there is empirical evidence that experience increases the foraging efficiency in other Hymenoptera. For example, in the wasps *Pepsis formosa* and *P. mildei* (Pompilidae) the time required for individual wasps to orient their bodies towards the host, to approach it and to complete the hunting sequence decreased during successive captures (Punzo 2005). As another example, short-term specialization of anthidiine bees (Apoidea) on one plant species increases foraging efficiency by reducing time and effort involved in learning to locate and manipulate flowers (Muller 1996). A similar increase in the efficiency of flower manipulation was also observed in bumblebees (Heinrich 1979).

Therefore, it is possible that individuals of *T. albonigrum* learn how to efficiently exploit specific spider genera and become specialized on them as a consequence. The hypothesis that this specialization increases foraging efficiency could be tested experimentally by measuring foraging efficiency (e.g. frequency of success in capture attempts, time required for immobilization) during successive feeding trials. Such experiments could also help us to discern if learning is more critical in searching for or handling spiders. An alternative approach would be to mark and follow individuals in the field and measure their foraging rate (a proxy for efficiency). If specialists are indeed more efficient, we would expect a correlation between specialization and foraging efficiency.

Interestingly, individual specialization in prey size followed an opposite trend from that of prey taxa, so that individuals in the wet season became more generalist in terms of prey size. The scenario that emerges from combining the data on prey size and taxa is that wasps rely mainly on *Eustala* in both the dry and wet seasons, but in the latter, although most individuals still prey on *Eustala*, part of them switch to previously under-utilized genera such as *Parawixia* and *Mangora*. Although the population as a whole becomes more generalist in the wet season, individuals stick to mainly one taxon, increasing interindividual variation. We speculate that the higher within-individual variance in prey sizes may be a reflection of a wider range of available prey sizes within each spider genera in the wet season, which needs to be investigated.

Temporal consistency

The timescale at which niche preferences persist is of utmost importance for the way that resource competition and frequency-dependent interactions will operate (Bolnick et al. 2003). For example, in *Pieris rapae* individuals specialize on a flower type during one day, based on their first encounter in that day, but they specialize on different flower types in successive days (Lewis 1986). In this case, individuals may switch quickly their food preferences in response to food abundance, and therefore there will be no frequency-dependent interactions. On the other hand, if diet specialization is linked to morphology (e.g. benthic and limnetic morphs in sticklebacks; Robinson 2000) competition and selection will be frequency-dependent and may have important evolutionary implications (Bolnick 2004). We hypothesize that *T. albonigrum* is somewhere in between these two extremes. Field observations indicate that individuals provide 1-2 cells per day (M.O. Gonzaga, unpubl. data). Since pipe-nest sets may contain up to 30 cells (Gonzaga & Vasconcellos-Neto 2005), nests probably correspond to many days' work of a given wasp, which would be a conservative estimate of the minimum timescale of individual consistency in this species. We are unaware of the number of nests built and provided by an individual wasp during its lifetime, but aggregations of contiguous nests (2 to 6; Fig. 1a) probably from the same individual are common (Gonzaga & Vasconcellos-Neto 2005). It would be of great interest to follow marked individuals during the construction of several nests, which would give us a better idea of the timescale of the foraging constancy in this species.

Ecological implications

An increase in the foraging efficiency of hunting-wasps, possibly due to their specializing on specific prey taxa, may have important implications. First, hunting-wasps must provide each cell with the necessary prey biomass for the full development of the larva. Therefore, a direct consequence of foraging more efficiently could be an increase in the number of cells a female will be able to provide before dying, which is ultimately a measure of its reproductive success. Second, wasps in the genus *Trypoxylon* suffer great pressure of parasitism by parasitoid flies (Bombyliidae, Sarcophagidae, Dolichopodidae, Phoridae) and wasps (Chrysididae, Ichneumonidae, Eulophidae) that oviposit on the larvae in the open cells left unattended while wasps are hunting for spiders(Gonzaga & Vasconcellos-Neto 2005). For instance, the percentage of larvae killed due to parasitism may reach as much as 41.3% in *Trypoxylon lactitarse* (Pérez-Maluf 1993) and 65% in *T. opacum* (Buschini & Wolff 2006). We have no information on the death rates of *T. albonigrum* larvae caused by parasitoids, but parasitoid flies were often seen entering unattended cells in the field (M.O. Gonzaga, unpubl. data), even in the presence of a guarding male wasp. Therefore, the survival of larvae may depend heavily on how fast a female wasp can provide a cell and close it. If this is true, individual specialization, by decreasing foraging time, may play a crucial role in reducing offspring mortality due to parasitism in *T. albonigrum* and possibly in other *Trypoxylon* species. A prediction that stems from this hypothesis is that the more specialized individuals in the population should have lower rates of offspring parasitism, which needs further investigation.

Conclusions

In a recent review of the incidence of individual specialization in natural populations, Bolnick et al. (2003) demonstrated the existence of individual-level diet variation in a wide range of animal taxa. Here, we present the first quantitative measure of individual specialization in wasps. The degree of individual specialization in *T. albonigrum* in the wet season (*IS* ~ 0.56) is among the strongest measures reported for natural populations (Bolnick et al. 2003). Future studies may reveal that individual specialization is actually a common feature of this taxonomic group. The studied individuals showed high consistency in their foraging preferences, even when the population exploited a variety of different prey taxa. Therefore, the studied population may be seen as a generalist population composed of individual specialists, which is in accordance with the presence of functional trade-offs constraining individual niches. The nature of the trade-offs remains unknown, but is likely to be behavioral. Future studies focusing on the ecology of this species, combining both descriptive and experimental approaches will certainly allow a better understanding of the mechanisms and implications of individual specialization in this system.

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Capítulo 4

Revisiting resource polymorphism in threespine stickleback: new insights from a complex network approach
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Ecology (aceito)

Revisiting Resource Polymorphism in Threespine Stickleback: New Insights from a Complex Network Approach

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ABSTRACT

Natural populations often exhibit variation for adaptation to alternate habitats and/or resources. For instance, in many north temperate lacustrine fishes, individuals vary in their propensity to use littoral versus pelagic habitats and prey. Such variation may give rise to discrete morphological groups. Alternatively, variation may be more quantitative, detectable by contrasting the morphology of individuals collected in divergent habitats. Here, we provide evidence for an even more subtle level of variation in a population of three-spine stickleback (*Gasterosteus aculeatus*). Based on complex network theory, we developed a quantitative index of inter-individual diet variation (E), and a measure of the degree to which a population is composed of discrete clusters of individuals with similar diets (C_{ws}). Using these measures, we re-analyzed a recent experimental study which showed that intraspecific competition led to elevated diet variation among individuals. Network analysis shows that this elevated diet variation occurred through increased clustering of individuals with respect to diet, and the addition of novel dietary clusters. Notably, we observe littoral/pelagic differentiation even when individuals were held in a homogenous habitat (9 m^2 enclosures, 2 m deep). Still more remarkably, we find that individual stickleback partition resources within littoral and within pelagic prey. Morphometric analysis reveals that these dietary clusters are morphologically differentiated groups. Thus, network-based approaches to quantifying and visualizing diet variation within populations provided evidence for a finer pattern of resource partitioning than the traditional “littoral-pelagic” dichotomy.

Key-words: optimal foraging theory; niche variation; individual specialization; resource polymorphisms; adaptive speciation; assortative mating; intraspecific competition; *Gasterosteus aculeatus*.

INTRODUCTION

Ecologists and evolutionary biologists have long been interested in understanding the forces that generate and maintain phenotypic variation in natural populations. Some of the most dramatic variation within natural populations entails ‘resource polymorphisms’, in which a single population contains discrete variation in morphological or behavioral phenotypes that exploit different habitats and/or resources (Robinson & Wilson 1994; Skúlason & Smith 1995; Smith & Skúlason 1996; Robinson 2000). This discrete variation provides an opportunity to study the ecological processes that promote variation, disruptive selection, and possibly speciation (Schluter & McPhail 1992; Schluter 1996; Snorrason & Skúlason 2004; Doebeli *et al.* 2007).

The existence of resource polymorphisms has been widely documented in multiple species of temperate fishes in postglacial lakes (Skúlason & Smith 1995; Smith & Skúlason 1996; Skúlason *et al.* 1999; Robinson & Schluter 2000). A clear pattern that emerges from these studies is the dichotomy between ‘benthic’ or ‘littoral’ morphs, and ‘limnetic’ or ‘pelagic’ morphs. Littoral morphs tend to be larger deeper bodied fish with fewer, shorter gill rakers, specialized on invertebrates of the littoral zone of the lake. Pelagic morphs are generally smaller, more streamlined fish with longer, more numerous gill rakers, and feed mainly on zooplankton in the pelagic zone of the lake (Robinson *et al.* 1996; Robinson 2000). In other cases, “morphs” have been defined based on distinctive feeding behavior (McLaughlin *et al.* 1994). For example, in young brook charr *S. fontinalis* there are sit-and-wait foragers specialized on crustaceans and actively-searching foragers specialized on insects (McLaughlin *et al.* 1994). Finally, morphs may also differ in life history traits, as for example in the arctic charr, *Salvelinus alpinus*, in which the four morphs differ greatly in size at sexual maturity (Snorrason *et al.* 1994).

Studies of resource polymorphisms in temperate fishes have relied primarily on two approaches to classifying individuals into different ‘morphs’. First, one can examine phenotypic distributions for discontinuities in morphological or life-history traits. A number of cases of resource polymorphisms exhibit strongly bimodal distributions, allowing one to easily assign individuals to a single morph *a posteriori* (Skúlason & Smith 1995; Smith & Skúlason 1996; Skúlason *et al.* 1999; Robinson & Schluter 2000). Alternatively, one can sample individuals from two or more different habitats, such as littoral and pelagic sites, and show that there are significant morphological differences (e.g., Robinson et al. 1996). In this case, the phenotype differences may be quite subtle, and morphs are defined *a priori* on the basis of capture location, rather than on the basis of morphology. Such subtle differences could arise from a bimodal distribution of habitat preferences, or simply from individual-level variation in habitat or prey preference (Bolnick et al. 2003), provided preference is at least weakly correlated with morphology. However, when behavioral or subtle morphological variation occurs within a given microhabitat, it becomes difficult to assign individuals to morphs, since there is neither a spatial basis for identifying *a priori* groups, nor a bimodal measurable trait. Consequently, many cases of within-habitat niche variation may have been overlooked because they do not rely on discrete morphological groups. Niche variation might therefore be more widespread than we currently appreciate ('individual specialization', Bolnick et al. 2003) because we lack the tools to properly identify and describe it.

In this paper, we provide evidence for diet variation among individual three-spine stickleback (*Gasterosteus aculeatus*), at a much finer scale than generally expected. In particular, we use a novel method based on network theory to re-analyze data from a recent experiment (Svanbäck & Bolnick 2007), and provide evidence for partitioning of littoral/pelagic prey in a phenotypically unimodal population. Our network method revealed clusters of individuals with broadly similar diets, yet non-overlapping with the diets of other clusters within the same enclosure. These clusters are morphologically distinct, and occurred both in wild-caught individuals, and among stickleback held within small experimental enclosures (9 m^2) that eliminated the effects of spatial variation on diet. Remarkably, our analysis also reveals a novel result that stickleback exhibit diet partitioning within littoral, and within pelagic prey. Finally, we use network theory to quantify levels of dietary clustering, and show that experimentally elevated intraspecific competition led to increased dietary clustering due to the addition of novel prey taxa as preferred prey became scarce. We conclude that the classical littoral-pelagic dichotomy may be insufficient to describe diet variation in lacustrine stickleback.

MATERIALS AND METHODS

Data collection

The data used in the present study comes from an experiment conducted in June 2005 in Blackwater Lake on northern Vancouver Island, British Columbia (Svanbäck & Bolnick 2007). For the sake of brevity, we will give a brief description of the data collection procedures and refer readers to Svanback & Bolnick (2007) for further details. Ten 9 m^2 enclosures made of 1/16 inch seine net were built, set in approximately two meter deep water, placed in pairs along 0.5 km of shoreline, and stocked with wild-caught sticklebacks to generate paired low- and high-density treatments (either 30 or 90 fish per enclosure; LD or HD hereafter). After 14 days, sticklebacks were trapped, anesthetized, and preserved in formalin. Stickleback were also sampled from outside each enclosure pair to serve as a natural baseline. Stomach contents were identified to the lowest feasible taxonomic level. Stomach contents provide a cross-sectional measure of an individual's diet, which may be biased if the forager is sampling from patchy prey, or if the stomach can only hold a few diet items at a time. However, stickleback guts usually contain many items, and the small scale of the enclosures ensured that all individuals were capable of sampling all available prey in much less time than it takes to digest them (> 6 hours). Consequently, the spatial scale makes it unlikely that the observed diet variation is the result of patchy resources or stochastic variation. In addition, significant correlations between morphology, stable isotopes, and diet suggest that diet variation is not due to stochastic sampling effects and can be a good guide to long-term differences in resource use (Bolnick et al, in review).

To test for associations between morphology and diet, four linear measurements were taken from the fish, namely body length, mouth width, mouth height, and gill raker length. The fish were photographed, and 23 homologous landmarks were digitized on the left side of each fish, and used in TpsRelw (Rohlf 2005) to convert the landmarks to partial warps and uniform scores.

Data analyses

The niche overlap network

A network is a representation of associations among elements in a system, in which nodes represent elements, and 'edges' are lines that connect those nodes that interact or are otherwise associated (Fig. 1). If we think of a network in terms of resource use, nodes represent individuals, and two individuals i and j are either connected by an edge if they use any resources in common, or disconnected if they do not share any resource type (Fig. 1). Weights can be assigned to the edges to represent the strength of the interactions among nodes. In the case of resource use, the strength of interactions can be equated to the degree of pairwise niche overlap and, therefore, this network depicts the degree of interindividual niche overlap (hereafter niche overlap network). We define w_{ij}, the pairwise niche overlap (adapted from Schoener 1968), as the measure of the degree of niche overlap between two individuals i and j:

$$w_{ij} = 1 - 0.5\sum_{k=1}^{K}\left|p_{ik} - p_{jk}\right| \qquad (1)$$

where p_{ik} is the frequency of category k in individual i's diet, and p_{jk} is the frequency of category k in individual j's diet. The proportion of the k-th resource category in individual i's diet, p_{ik} is calculated as:

$$p_{ik} = \frac{n_{ik}}{\sum_k n_{ik}} \tag{2}$$

where n_{ik} represent the number (or mass) of diet items in individual i's diet that fall into category k. Numbers of prey consumed may be more appropriate for behavioral ecologists studying prey capture decisions, whereas mass may be more appropriate for studies of energy flux through a community or competition. The pairwise niche overlap ranges from 0 (no overlap) to 1 (total overlap). We therefore define w_{ij} as the weight of the edge that connects the individuals i and j (Fig. 1).

Measuring the degree of interindividual niche variation

First, we define O, the summation of the total pairwise overlap:

$$O = \sum w_{ij} \tag{3}$$

In a network composed of individuals whose diets are identical (no interindividual niche variation), all individuals are connected and $w_{ij} = 1$ for all pairs of individuals. In such a network, $O = n(n-1)/2$, which corresponds to the number of edges of a completely connected network with n individuals. We therefore need to standardize total pairwise overlap by the number of potential edges, yielding a measure of the degree of interindividual niche similarity:

$$\breve{O} = \frac{O}{n(n-1)/2} = 2O/n(n-1)\,. \tag{4}$$

a

	Resource 1	Resource 2	Resource 3
Individual 1	24	0	0
Individual 2	48	0	0
Individual 3	20	0	0
Individual 4	1	112	0
Individual 5	0	14	0
Individual 6	0	70	0
Individual 7	0	1	39
Individual 8	0	0	102
Individual 9	0	0	219

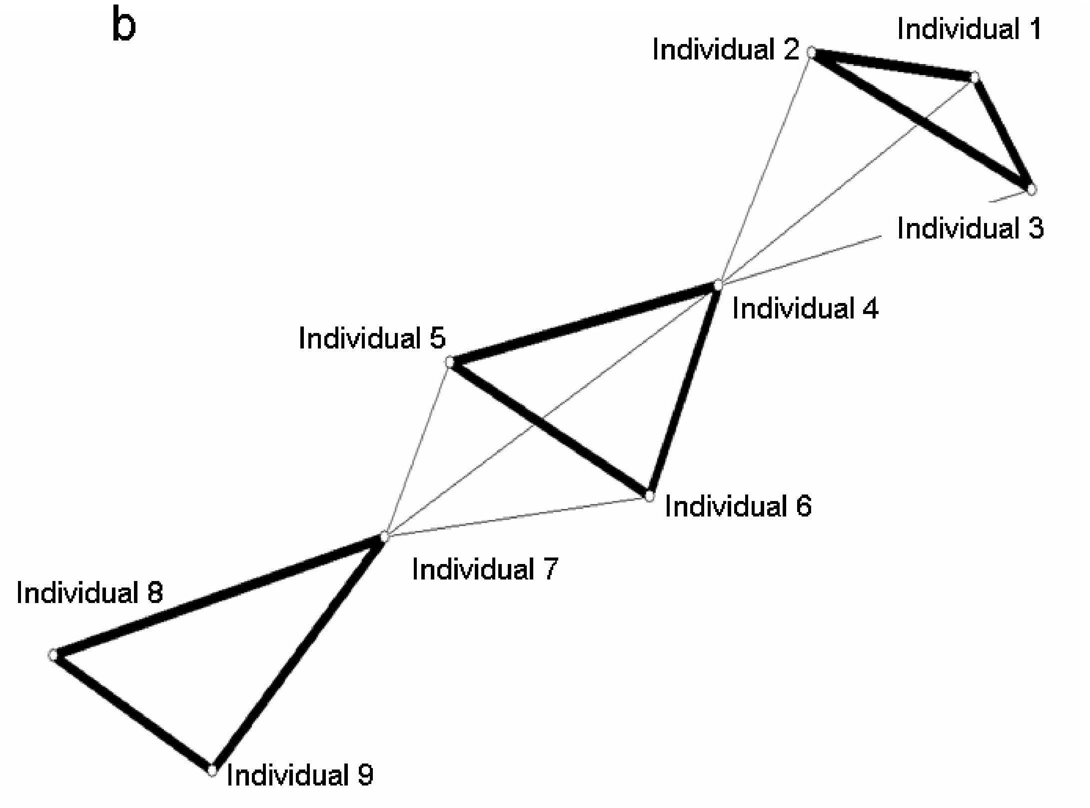

Fig. 1. The use of resources by different individuals of a hypothetical population described as a matrix **a**, and a network **b**. The widths of links are proportional to the degree of pairwise overlap between individuals (w_{ij}). Absence of an edge between two individuals indicates no niche overlap ($w_{ij} = 0$). This network approach allows the description of previously unexplored, intrapopulation structures, such as clusters (see text for details). For example, the depicted hypothetical population has three clusters formed by individuals 1-2-3, 4-5-6, and 7-8-9.

$\breve{O}$ ranges from 1 when there is no niche variation, towards 0 as variation increases. We define a second index which is positively related to niche variation,

$$E = 1 - \breve{O}$$

and ranges from zero (no variation) to 1 (no overlap among any pairs of individuals). The index $\breve{O}$ has known statistical properties that allow us to calculate the variance of $\breve{O}$ and E (Appendix 1). One can use these variances to determine confidence intervals, conduct parametric tests of the null hypothesis of no diet variation, and statistically compare the levels of diet variation between multiple populations. E thus offers some important advantages over previous indices of diet variation (Bolnick et al. 2002).

Particularly with cross-sectional gut-content data, apparent diet variation may arise simply from individuals stochastically sampling diet items from a shared population distribution (Bolnick et al. 2002). It is thus important to test the null hypothesis that the observed $E > 0$. We used both *t*-tests using calculated variances (Appendix 1), and a non-parametric test of this null. For non-parametric tests, each individual was reassigned the same number of prey items that it was observed eating, drawn randomly from the population diet distribution via multinomial sampling (Bolnick et al. 2002). Calculating E for each resampled population (we used 10,000 iterations), the null hypothesis can be rejected if the observed E is greater than 95% of the null values.

We developed a program in C language, DIETA1 (see Supplement), to calculate E and to perform Monte Carlo simulations. We calculated E for the wild-caught control stickleback (caught outside the enclosures), and for fish from within each enclosure. Two-tailed one-sample *t*-tests were used to contrast the LD and HD treatments against the control.

Measuring the degree of clustering

The concept of clustering reflects the degree to which nodes are organized into highly connected subgroups (clusters) that are weakly connected to each other. A clustering index compares the overall density of connections to the density of connections at individual nodes (Fig. 1B). When analyzing diet data, clustering occurs when the population is organized into discrete groups of individuals sharing a common set of resources, overlapping little with other groups of individuals. Clustering also implies that there are few dietary intermediates (e.g., few individuals consume roughly equal quantities of littoral and pelagic prey). In binary networks, the density of connections is measured in terms of the number of connections (Watts & Strogatz 1998), but since we are dealing with weighted networks the density of connections here refers to both the number and weight of connections. We propose measuring niche clustering with a weighted clustering coefficient, C_w (Barrat et al. 2004). The weighted clustering coefficient of individual i, C_{w_i}, is a combined measure of the number and weight of the edges around individual i and among the nodes directly connected with i defined as:

$$C_{w_i} = \frac{1}{s_i(k_i - 1)} \sum_{j,h} \frac{(w_{ij} + w_{ih})}{2} a_{ij} a_{ih} a_{jh} \qquad (6)$$

where s_i is the sum of the weights (w_i) of all the edges between individual *i* and the individuals to which it is connected; k_i is the number of edges between individual *i* and the individuals to which it is connected; w_{ij} is the weight of the edge between individual *i* and *j*; w_{ih} is the weight of the edge between individual *i* and *h*; and a_{ij}, a_{ih}, and a_{jh} are 1 if an edge is present between each pair *ij*, *ih*, and *jh*, and zero otherwise. The network weighted clustering coefficient, C_w, is the average value of the individual clustering coefficients, C_{w_i}, for all nodes in the network.

One important feature of C_w is that it is directly proportional to the average network density of connections (measured by $\breve{O}$), so that $C_w \sim \breve{O}$ in a totally random network (in our case, a network consisting of individuals that sample randomly from the population niche). This means that two random networks will differ in their measures of C_w simply if they differ in their values of $\breve{O}$. As a consequence, directly using C_w as a measure of the degree of clustering may be misleading, especially if one wants to compare different networks. As a way to circumvent this problem, we define C_{ws}, which is a correction of C_w that controls for the effect of $\breve{O}$:

$$C_{ws} = (C_w - \breve{O}) / \breve{O} \tag{7}$$

Now, the degree of clustering is measured relative to $\breve{O}$, and in a totally random network $C_{ws} \sim 0$. An interesting feature of C_{ws} is that it can assume both positive and negative values. C_{ws} will be positive ($C_w > \breve{O}$) if the local density of connections is higher than the overall density of connections, indicating that the population is characterized by clusters of individuals sharing common resources (Fig. 1B). In contrast, C_{ws} will be negative ($C_w < \breve{O}$) when the local density of connections is lower than the overall density of connections, indicating that individuals usually use a very particular combination of resources that differs from that of other individuals (i.e. individuals' diets are overdispersed).

A null model approach similar to that described for E can be used to test the significance of this index. The main difference now is that the test performed is two-tailed. If $C_{ws} > 0$ and higher than 97.5% of the null C_{ws} values, there is significant clustering in the analyzed network. On the other hand, if $C_{ws} < 0$ and C_{ws} is lower than 97.5% of the null C_{ws} values, there is significant evidence of overdispersion. We used DIETA1 to calculate C_{ws} and their corresponding P-values for all enclosures and the sample of wild-caught control fish. In order to compare the degree of clustering between treatments we did two-tailed one-sample t-tests to contrast the C_{ws} measures in the LD and HD treatments against the sample of wild-caught control fish.

Assigning individuals to discrete groups

After detecting significant clustering, one can determine individuals' membership in clusters. One can then ask additional questions about morphological, behavioral, genetic, or fitness differences between post-hoc clusters (as opposed to the *a priori* approach used when sampling individuals from multiple habitats). Clusters can be defined in several different ways (e.g. *cliques*, *k-cores*, *k-plexes*; Wasserman et al. 1994). Unfortunately, present methods for defining clusters rely on presence/absence of connections, rather than weights. As a result, very weak diet overlap between two individuals (low w_{ij}) would be treated as equivalent to total diet overlap ($w_{ij} = 1$) when assigning individuals to clusters. To circumvent this drawback, we adopted an approach that has been widely used in the network literature and relies on the definition of a cut-off value that defines strong edges in a given network (e.g. Costa 2004). We defined a strong edge as one whose w_{ij} is higher than the population average pairwise overlap ($w_{ij} > \breve{O}$). The simplest concept of a cluster is the *clique*, which is defined as a group of nodes in which all nodes are connected to each other. We defined a *w-clique* as a *clique* in which nodes are interconnected by the so-defined strong edges. Following the identification of *w-cliques*, we determined the dominant prey taxa that characterized each clique.

We used DIETA1 to generate weighted and binary matrices that can be imported into commonly used programs of network analyses. These programs allow not only the drawing of the networks, but also the identification of the *w-cliques*. We used the program Pajek (Batagelj & Mrvar 1998), free available for download at http://vlado.fmf.uni-lj.si/pub/networks/pajek, to draw networks and to assign individuals to *w-cliques* in all enclosures and wild-caught fish.

Morphological analyses

Having identified discrete clusters of highly similar individuals (see Results), we wanted to know whether these clusters are morphologically distinguishable. For the sake of statistical power, we pooled the data from all enclosures according to treatment, so that we ended up with three datasets: control, low density, and high density. We assigned individuals within each treatment to different diet groups, which we determined using the network analyses of diet data. To compare the morphology among the diet groups we used two types of variables (body length, mouth width, mouth height, and gill raker length), linear distance measures and geometric shape variables. Geometric shape variables (partial warps; Bookstein 1991) were derived from morphological landmarks archived as two-dimensional Cartesian coordinates. Patterns of morphological variation among diet groups were assessed by canonical variate analysis for linear distances, standardized linear distances (corrected for body length) and landmark data. Ninety-five percent confidence regions around centroids for canonical axes were constructed using parametric bootstrap theory (Ringrose 1996; Von Zuben *et al.* 1998). Body form changes were visualized as deformations by using the TpsRegr program (Rohlf 2000).

RESULTS

Patterns of resource use

We found significant interindividual diet variation within each of the ten 9 m^2 enclosures, and in the wild-caught fish (Table 1). The *t*-tests using the calculated variances for E (Table 1) indicated that the observed E-values were larger than zero in all samples ($P < 0.0005$), in agreement with the non-parametric tests (Table 1). We also found significant clustering in the control and in all but one LD enclosure (Table 1). In the LD treatment, the mean degree of interindividual diet variation ($E = 0.687$) and mean degree of clustering ($C_{ws} = 0.308$) did not differ from the control (Table 1; E: $t_4 = 0.393$; $P = 0.714$; C_{ws}: $t_4 = 0.209$; $P = 0.844$). On the other hand, in the HD treatment, the mean E (0.786) and C_{ws} (0.466) were significantly higher than the control (Table 1; E: $t_4 = 6.073$; $P = 0.004$; C_{ws}: $t_4 = 3.073$; $P = 0.037$). We conclude that increased competition in the HD-treatment led to increased interindividual diet variation, as shown previously in the original analysis by Svanbäck and Bolnick (2007). However, our clustering measure adds the novel insight that diet variation arose via increased clustering. Note that since the clustering coefficient controls for level of diet variation (E), this increased clustering is not redundant with our finding of increased diet variation.

Table 1: The E measure of interindividual diet variation and the C_{ws} measure of clustering in wild-caught, low-density (LD), and high-density (HD) treatments in a population of threespine sticklebacks, *Gasterosteus aculeatus*. N: sample size; σ_E: standard deviation of E; P-values were estimated with Monte Carlo bootstraps (10,000 replicates).

Enclosure #	Pair	Treatment	N	E	σ_E	P	C_{ws}	**P**
1	A	LD	12	0.5959	0.00929	< 0.001	0.0194	0.2029
2	A	HD	49	0.7580	0.00039	< 0.001	0.3218	< 0.001
3	B	HD	39	0.7300	0.00058	< 0.001	0.3496	< 0.001
4	B	LD	22	0.7060	0.00321	< 0.001	0.4163	< 0.001
5	C	HD	44	0.8081	0.00030	< 0.001	0.5875	< 0.001
6	C	LD	21	0.7225	0.00231	< 0.001	0.4554	< 0.001
7	D	LD	16	0.7321	0.00687	< 0.001	0.3778	< 0.001
8	D	HD	48	0.8205	0.00037	< 0.001	0.4799	< 0.001
9	E	LD	23	0.6777	0.00272	< 0.001	0.2692	< 0.001
10	E	HD	45	0.8155	0.00047	< 0.001	0.5888	< 0.001
Wild-caught	–	control	52	0.6772	0.00078	< 0.001	0.2912	< 0.001

In four of five enclosure pairs, the HD enclosures exhibited more clustering than their LD counterparts (*E* was greater in all cases, Table 1). The tendency towards higher clustering in the HD treatment was confirmed by visual inspection of the niche overlap networks. For the sake of brevity, we only show the networks for one pair of enclosures (Table 1; pair C) to illustrate this trend (Fig. 2). It is important to notice that the empirical networks in both LD and HD treatments (Fig. 2A and C) were strikingly more clustered than null networks, in which individuals sample randomly from the population diet (Fig. 2B and D).

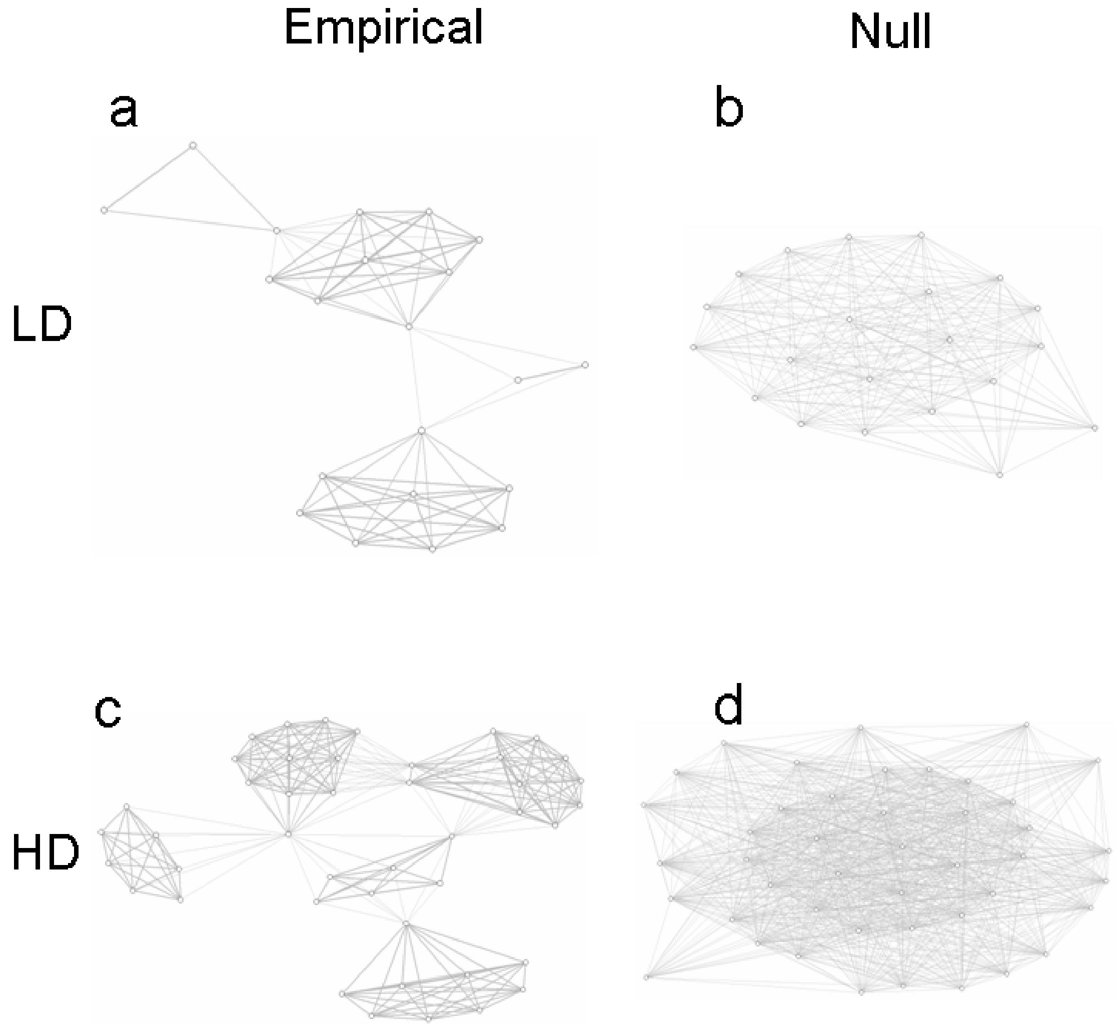

Fig. 2. Weighted networks representing the pair C of enclosures (Table 2): **a** and **c** empirical networks of the low- and high-density enclosures, respectively; **b** and **d** null networks corresponding to the median C_{ws} value of a null distribution of 10,000 C_{ws} values generated by a Monte Carlo procedure. Note that the empirical networks are strikingly more clustered than their null counterparts. Individuals are connected if they consumed resources in common. The strength of the edges is a measure of the degree of pairwise niche overlap among individuals.

Although the visual identification of clusters was straightforward in most cases, as illustrated in the previous example, we needed to identify the *w-cliques* within networks in order to objectively determine the affiliation of individuals to diet groups. The number of *w-cliques* varied from two to five (Table 2) and was consistently larger in the HD than in the LD treatments (one-tailed paired *t*-test: $t_4 = 5.880$; $P = 0.002$). For example, in the pair C, we found two *w-cliques* in the LD enclosure and five *w-cliques* in the HD enclosure (Fig. 3). In this pair, in the LD enclosure, one *w-clique* consumed preferentially littoral macroinvertebrates and the other, pelagic cladocerans (Fig. 3A), thus corresponding to the classic littoral/pelagic dichotomy. The overall results (Table 2) indicate that littoral macroinvertebrates were the preferred resource in the LD treatment, followed by pelagic cladocerans and chironomids. In the HD enclosure, these two resources again represented cliques, but many individuals resorted to novel resources, forming three additional cliques using pelagic macroinvertebrates, chironomids, and benthic cladocerans (Fig. 3B).

Table 2: Number of identified *w-cliques* and number of individuals assigned to each *w-clique* in wild-caught, low-density (LD), and high-density (HD) treatments in a population of threespine sticklebacks, *Gasterosteus aculeatus*. *w-cliques* (groups in which all individuals are connected to each other by strong connections) were classified according to diet: LM (littoral macroinvertebrates); PC (pelagic cladocerans); PM (pelagic macroinvertebrates); Ch (chironomids); and BC (benthic cladocerans).

Enclosure #	Pair	Treatment	# of *w-cliques*	***# of individuals within* w-cliques**				
				LM	PC	PM	Ch	BC
1	A	LD	2	6			5	
2	A	HD	4	10	14		6	11
3	B	HD	3	12	11			9
4	B	LD	2	10	6			
5	C	HD	5	10	9	7	6	10
6	C	LD	2	7	3			
7	D	LD	2	8	3			
8	D	HD	5	11	7	8	7	8
9	E	LD	3	10	5		3	
10	E	HD	5	11	6	6	7	12
Wild-caught	–	control	3	21	15	4		

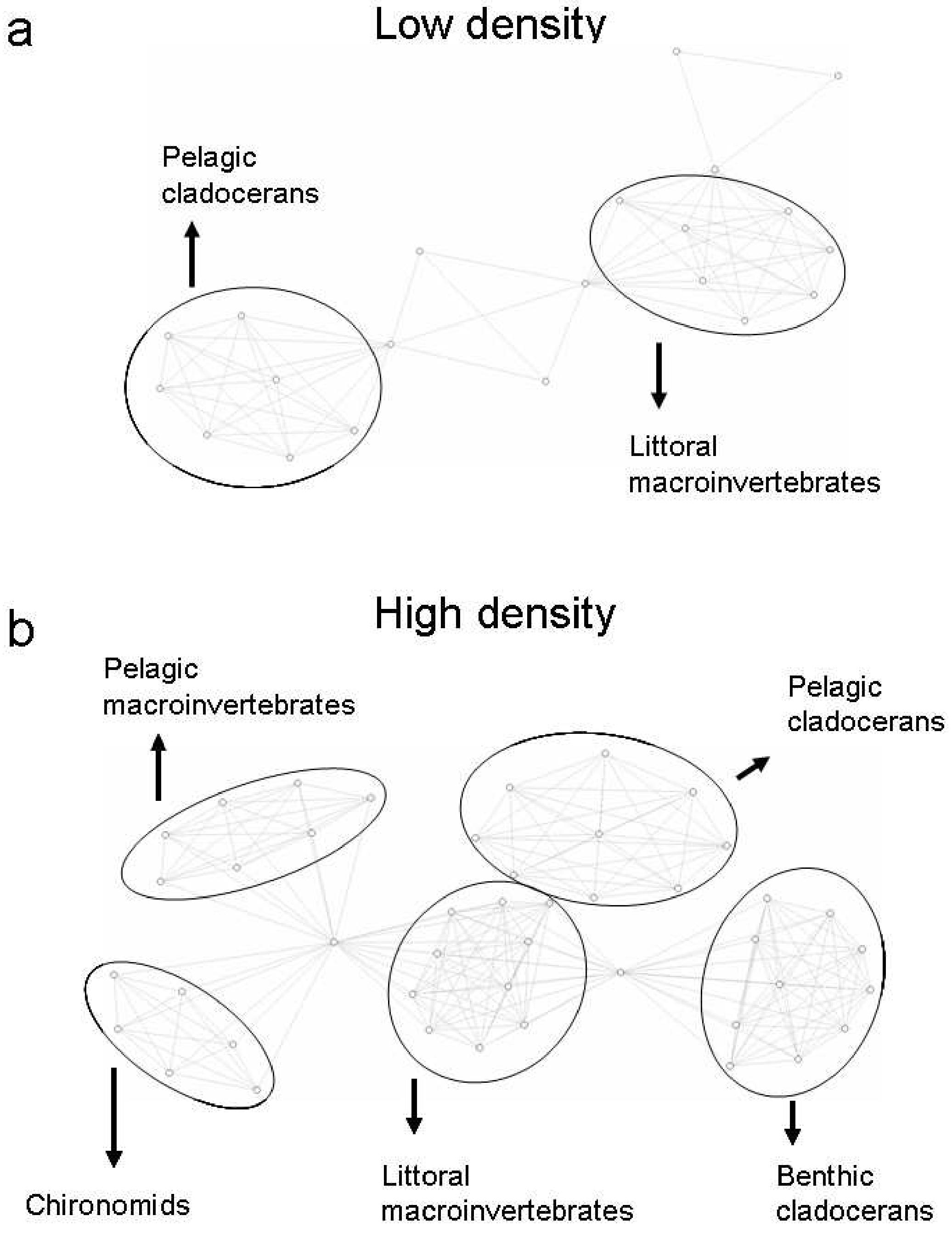

Fig. 3. Binary networks of strong connections extracted from the networks depicted in Fig. 2, showing their *w-cliques* (a group in which all individuals are connected to each other) and the resources consumed by each *w-clique*. The resource type consumed by one *w-clique* was not consumed by another *w-clique* in the same network: **a** low-density enclosure showing two *w-cliques*; **b** high-density enclosure showing five *w-cliques*. A higher number of *w-cliques* in the high-density treatment was a general trend among enclosure pairs.

Morphology vs. diet

The morphological distribution in Blackwater Lake stickleback was unimodal, with no indication of discontinuities that would indicate distinct resource polymorphisms (Fig. 4). Nevertheless, the post-hoc *w-cliques* revealed previously unrecognized diet-morphology associations. After pooling the data for all enclosures within each treatment, we ended up with three diet groups in the wild-caught fish, namely, littoral macroinvertebrate eaters (LM-eaters), pelagic cladoceran eaters (PC-eaters), and pelagic macroinvertebrate eaters (PM-eaters). In the LD treatment, there were also three diet groups, one feeding on littoral macroinvertebrates, one feeding on pelagic cladocerans, and one feeding on chironomids (Ch-eaters). Finally, in the high density treatment, we found five diet groups, four of which were also present in the other treatments and a novel group feeding on benthic cladocerans (BC-eaters). We stress that these groups occur repeatably within enclosures (Table 2), and are not a result of between-enclosure variation.

In the wild-caught fish, the analyses of linear measurements showed a clear separation between individuals feeding on littoral macroinvertebrates on one side, and those feeding on pelagic prey on the other side (Figure 5a). However, there was no difference between individuals feeding on pelagic cladocerans and pelagic macroinvertebrates (Figure 5a). As expected, the pelagic feeders were smaller, had smaller mouths and longer gill rakers compared to the littoral feeders (Figure 5b).

In the LD treatment, we still observed the two main diet groups, LM-eaters and PC-eaters (Figure 5c), and a novel littoral group, Ch-eaters (Figure 5c). We still see a clear separation between the morphologies of LM- and PC-eaters, whereas Ch-eaters

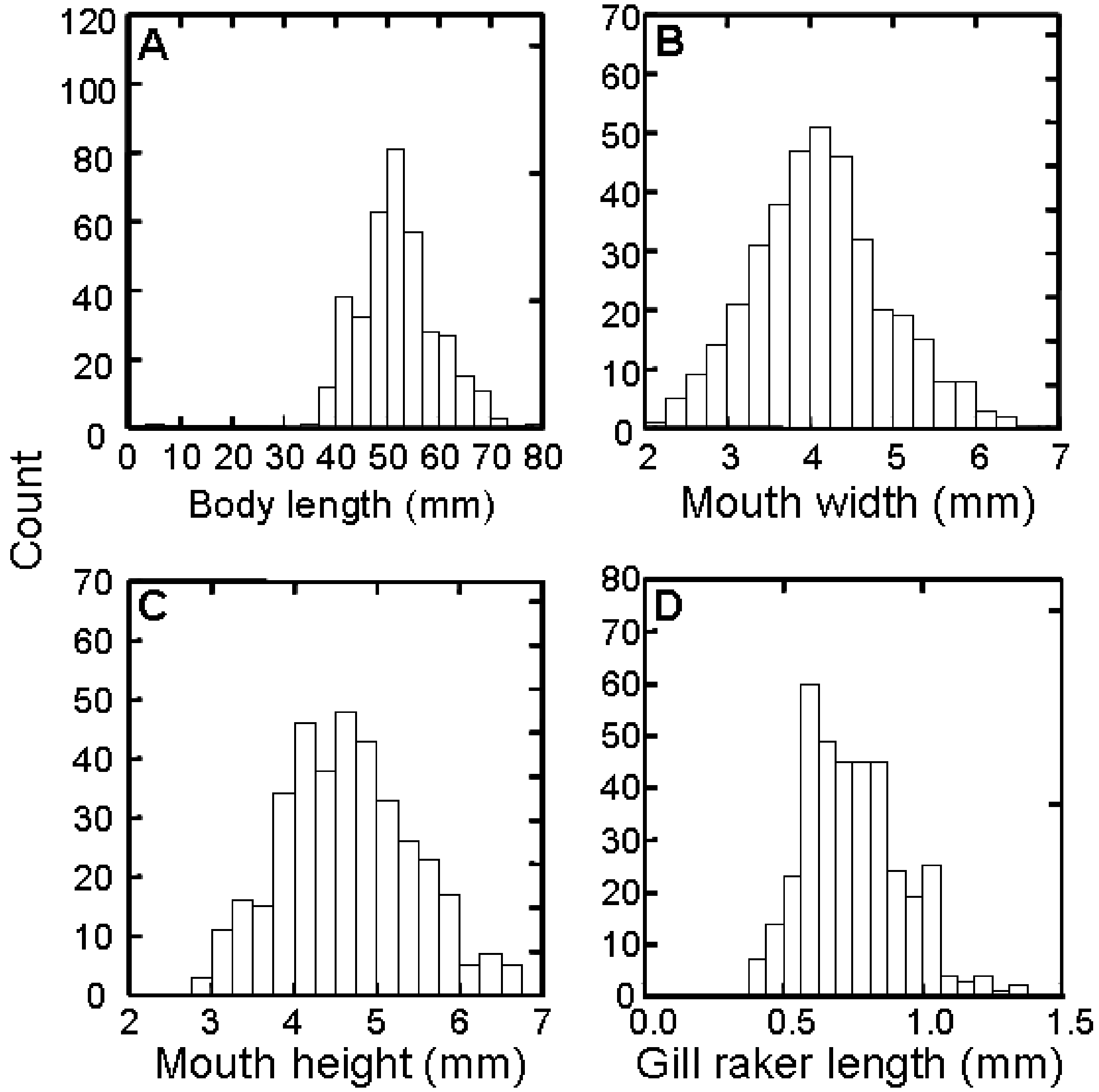

Fig. 4. Frequency distributions of linear measurements of four morphological variables of a population of threespine sticklebacks, *Gasterosteus aculeatus*, in Blackwater Lake, on northern Vancouver Island, British Columbia. Morphological variables are: **a** body length; **b** mouth width; **c** mouth height, and **d** gill raker length. Note that the morphological distribution is unimodal with respect to all four variables, allowing no *a priori* identification of discrete groups. *N* = 370 fish.

showed an intermediate morphology between the two extremes (Figure 5c). Due to the small sample size (8 individuals), however, the Ch-eater confidence ellipse was very large and overlapped greatly with the other ellipses, hindering further interpretation

(Figure 5c). Again, pelagic feeders showed the typical morphological syndrome of smaller body and mouth, and longer gill rakers (Figure 5d).

In the HD treatment, we found that individuals eating littoral macroinvertebrates and chironomids were morphologically very similar, being larger, larger-mouthed and having shorter gill rakers than individuals feeding on cladocerans and pelagic macroinvertebrates (Figure 5e, f). Interestingly, BC-eaters, although feeding on a benthic resource, had a 'pelagic' morphology, which makes sense if these cladocerans, in spite of being benthic, are small-sized and more similar in morphology to pelagic than to other benthic prey (Figure 5e). There was a clear separation on canonical variate 2 between the fish that ate cladocerans and those that ate pelagic macroinvertebrates, indicating a subtler morphological differentiation than that associated with the major littoral-pelagic resource axis (Figure 5e). The patterns described above also held in the analyses using the standardized linear data and are not shown.

We could not use the partial warps for the wild-caught and the LD treatment because of singular data matrices. Therefore, we present only the results for the HD treatment. We found a major separation between cladoceran-eaters and littoral-prey eaters, with PM-eaters showing an intermediate morphology (Fig. 6). More important, within each of these major groups, there was a clear separation between diet groups on canonical variate 2. As we can see from the deformation grids (Fig. 6), individuals with higher scores on both axes 1 and 2 had a more pelagic morphology, tending to be more slender and to have a more pointed snout, as opposed to the LM- and Ch-eaters, which had a more littoral morphology with a deeper body and a more blunt snout (Fig. 6).

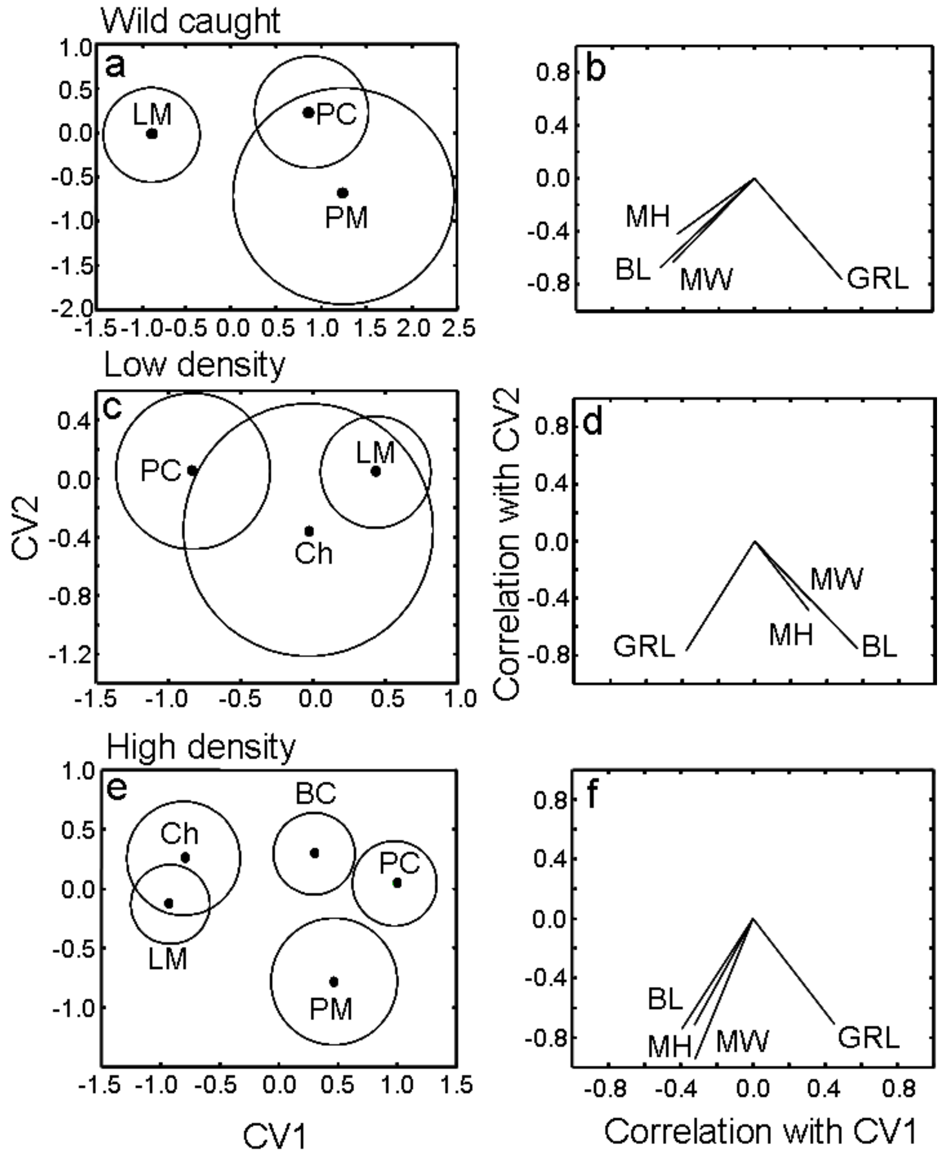

Fig. 5. Morphological variation among diet groups within wild-caught fish, low-, and high-density treatments, based on four linear measurements: **a**, **c**, and **e** bivariate plot of centroids (denoted as dots) and 95% confidence regions for canonical variates 1 and 2 (CV1 and CV2, respectively) derived from linear measurements. **b**, **d**, and **f** vectors portraying the principal directions of variation (estimated as Pearson correlation coefficients) in linear measurements in the plane of the first two canonical variates. Diet groups were: littoral macroinvertebrates (LM), pelagic cladocerans (PC), pelagic macroinvertebrates (PM), chironomids (Ch), and benthic cladocerans. Linear measurements were: body length (BL), mouth height (MH), mouth width (MW), and gill raker length (GRL). Confidence regions derived from parametric bootstrap. Percentage of variance explained by CV1 and CV2, respectively: **a** 92.28% and 7.72%; **c** 95.22% and 4.78%; **e** 83.65% and 14.23%. No overlap of confidence regions indicates significant morphological differences.

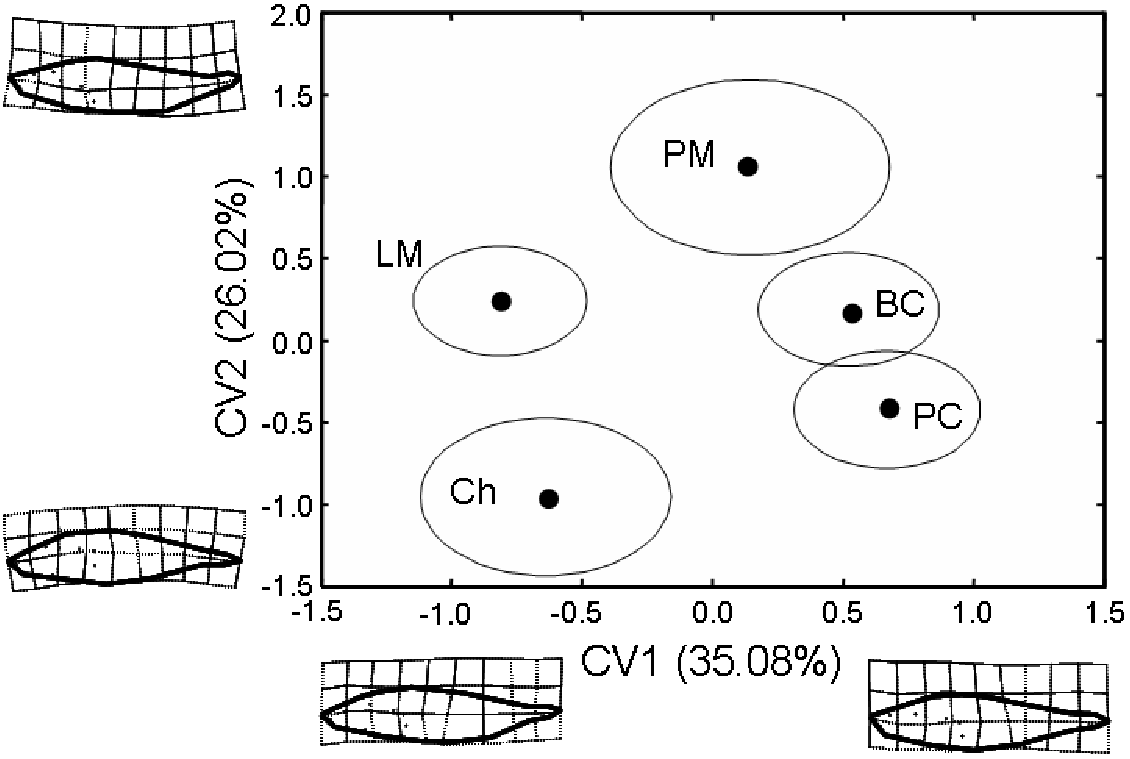

Fig. 6. Bivariate plot of centroids (denoted as dots) and 95% confidence regions for canonical variates 1 and 2 (and percent variance explained) derived from a canonical variate analysis of partial warp scores of coordinate landmark data. The deformation grid plots below and to the left of the graph are estimated changes in body shape implied by the first and second canonical variates for positive and negative deviations from the mean shape (consensus). Confidence regions derived from parametric bootstrap. No overlap of confidence regions indicates significant morphological differences. The deformation grid plots were exaggerated (3×) to make the visualization easier.

DISCUSSION

In a previous study, Svanbäck and Bolnick (2007) reported that resource competition led to increased diet variation among individuals. By reanalyzing their data using our newly developed indices, we were able to gain new insight into the diversifying effect of intraspecific competition. In particular, we found that diet variation increased in high density treatments because some individuals switched to form novel dietary groups using previously rarely-used prey, resulting in increased dietary clustering. Since morphological variance was equal in high and low density treatments (Svanbäck & Bolnick 2007), this increased clustering reflects behavioral changes rather than increased morphological variation. Nevertheless, we also found significant morphological differences among dietary clusters. We were thus able to identify diet variation that is behaviorally flexible but linked to morphology, despite the lack of discrete morphological groups. In the following discussion, we consider i) the theoretical basis for this behavioral flexibility, and ii) the evolution and maintenance of polymorphisms.

Optimal foraging theory

Our results are consistent with the predictions of a simple model of optimal foraging theory (OFT), in which phenotypes share a single preferred resource, but resort to different alternate prey when the preferred resource becomes scarce (Robinson & Wilson 1998; Svanbäck & Bolnick 2005). In such a scenario, competition is expected to increase both the population niche width and interindividual diet variation, because increases in the population niche width should outpace increases in individual niche width (Svanbäck & Bolnick 2005). This theoretical prediction was subsequently confirmed (Svanbäck & Bolnick 2007) by the stickleback experiment that we re-analyze here.

Our new index allowed us to identify the preferred and alternative resources to which individuals resort under high competition ("competitive refuge"; Svanbäck & Bolnick 2005). In the stickleback studied, littoral macroinvertebrates seem to be the preferred resource, being consumed in all LD enclosures and being a major diet item among the wild-caught fish (Table 2). Pelagic cladocerans seem to be the second most important resource, being heavily consumed by the wild-caught fish and also in most of the LD enclosures (~ 30% of individuals). This is in accordance with the major littoral-pelagic dichotomy already described for sticklebacks (Schluter & McPhail 1992) and other lacustrine fishes (Skúlason *et al.* 1999; Robinson & Schluter 2000). This dichotomy has been interpreted as resulting from strong trade-offs associated with the use of littoral and pelagic resources (Schluter 1995; Robinson 2000). Our results revealed that under high competition littoral and pelagic resources are more finely partitioned, with groups of morphologically different individuals seeking refuge in different alternative resources, namely, pelagic macroinvertebrates, chironomids, and benthic cladocerans. This suggests the possibility of unknown trade-offs in resource use within the littoral and pelagic habitats that would become important under high competition. Consistent with this inference, we found significant morphological differences among littoral dietary clusters, or among pelagic clusters. We therefore conclude that the broad categorization of resources as "littoral" or "pelagic" hides finer subdivisions that are perceived by sticklebacks at times of resource limitation.

Evolution of resource polymorphisms

Discrete resource polymorphisms have been widely documented in nature (Skúlason & Smith 1995; Smith & Skúlason 1996) and more specifically in temperate lacustrine fishes (Skúlason *et al.* 1999; Robinson & Schluter 2000). When polymorphisms have a morphological basis so that morphotypes can be identified *a priori*, the description of polymorphisms is straightforward. For example, the arctic charr, *Salvelinus alpinus*, has four greatly distinct morphs, two occupying the littoral zone and two the pelagic zone of the lake (Snorrason *et al.* 1994). There are cases, however, in which the basis of resource polymorphisms are either behavioral or related to life-history traits (McLaughlin *et al.* 1994; Snorrason *et al.* 1994; Smith & Skúlason 1996), so that the detection of polymorphisms is greatly hindered (Skúlason & Smith 1995). Our method, by looking directly at the patterns of resource use among individuals, can improve our ability to detect cases of resource polymorphism in which phenotypic discontinuities do not occur. We suggest that applying our method to other cases of littoral/pelagic polymorphisms might reveal more subtle niche variation and partitioning within habitats.

One hypothesis for the evolution of resource polymorphisms in post glacial fishes posits that colonization of a new lake is quickly followed by niche variation via plastic foraging behavior. Following colonization and population growth, increasing intraspecific competition for preferred resources would favor diversification onto novel prey (Skúlason *et al.* 1999; Snorrason & Skúlason 2004). This diversification would be facilitated not only by the presence of a variety of resources, but also by the low interspecific competition typical of depauperate post glacial lakes (Robinson *et al.* 1993; Snorrason & Skúlason 2004). Depending on genetic and developmental mechanisms and the mating system, this variation may become associated with plastic or with genetic morphological variation. Depending on the strength of trade-offs and divergent selection, assortative mating, or the genetic complexity of morphological traits, discrete forms may appear (Robinson & Wilson 1994), or possibly sympatric species (Dieckmann & Doebeli 1999). Several hypothesis concerning the evolution of resource polymorphisms stem from this theory. For instance, we would expect that more heterogeneous environments (either structurally or with more variable resources) should harbor higher degrees of polymorphisms. Additionally, more depauperate communities with fewer competitors or predators, in which populations experience ecological release, should also be associated with higher degrees of polymorphisms. Our method provides a quantitative framework that can be used to test these predictions at the level of more subtle diet variation, which could help us to better discern the ecological conditions that favor the evolution of resource polymorphisms in natural populations.

The morphological variation of postglacial fishes has so far been described in terms of 'littoral' and 'pelagic' morphs representing either the ends of a continuous variation (Robinson 2000) or discrete entities (Schluter & McPhail 1992; Snorrason *et al.* 1994; Skúlason *et al.* 1999). In the present paper, we document morphological and dietary differences among groups of individuals that go beyond the classical littoral and pelagic syndromes. The association between morphology and diet seems to be mediated by behavior in this system (Svanbäck & Bolnick 2007) emerging only under high-competition regimes. An interesting question is to what extent the observed morphological variation results from phenotypic plasticity (Schluter 1995; Robinson *et al.* 1996) or is genetically determined.

Finally, the degree to which the individuals within a population are sorted into dietary clusters, or 'microguilds', may have ecological and evolutionary implications. Few models have explicitly examined the community-level effects of within-population diet variation in general (Doebeli 1997), let alone clustering in particular. We therefore do not know how the degree of clustering might influence food web dynamics. The implications for intraspecific competition, however, are quite intriguing. Most theoretical models of intraspecific competition assume that competition between individuals falls away continuously with phenotypic difference (Roughgarden 1972; Taper & Case 1985;

Dieckmann & Doebeli 1999). The existence of discrete microguilds suggests that individuals will either tend to compete strongly, or not at all. The degree of clustering thus may dictate the types of models one should use for studies of frequency-dependent intraspecific competition.

Frequency-dependent competition plays a major role in driving disruptive selection (Bolnick 2004), which may drive additional evolutionary diversification. Such diversification can in theory include speciation, which also requires strong assortative mating (Dieckmann & Doebeli 1999). Interestingly, clustering might provide a viable basis for assortative mating, if mate preferences are influenced by diet similarity. Assortative mating by diet might arise 1) via spatial segregation into different microhabitats, 2) temporal isolation if prey availability peaks at different times of year for different groups, or 3) via direct mate choice, for instance if individuals prefer to school with conspecifics with more similar diets. The latter effect has recently been demonstrated in stickleback (Ward *et al.* 2004; Ward *et al.* 2005). In conclusion, the degree clustering is likely to influence the potential for evolutionary divergence in natural populations.

Conclusions

In this paper, we have introduced a new approach, based on complex networks, to study resource polymorphisms. The study of resource polymorphisms offers an opportunity to understand the ecological and evolutionary forces that generate and maintain phenotypic and genetic variability in natural populations. Our method allowed the identification of an undocumented pattern of intra-population variation in resource use in threespine sticklebacks, in which individuals form microguilds that represent a finer partition of the broad "littoral" and "pelagic" resource categories. We believe that our approach provides a new, useful tool that may yield deeper insights into the evolution of resource polymorphisms. At the very least, our results call for a reappraisal of the patterns of resource use in sticklebacks and, possibly, post-glacial lacustrine fishes in general, and for a renewed effort to understand the mechanisms underlying the evolution of within-population niche variation.

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APPENDIX 1

Derivation of the variance of *E*.

A Jackknife estimation of the variance of $\breve{O}$ can be derived using the formalism of *U*-statistics (Arversen 1969). We first note that

$$\breve{O} = \binom{n}{2}^{-1} \sum_{1 \le i \le j \le n} w_{ij} \tag{1}$$

i.e. $\breve{O}$ is a *U*-statistic of degree 2 and kernel given by:

$$w_{ij} = 1 - 0.5 \sum_{k=1}^{K} \left| p_{ik} - p_{jk} \right| \tag{2}$$

It is asymptotically normal with mean θ and variance $4\xi_1^2$ for

$$\theta = Ew_{12} = 1 - 0.5 \sum_{k=1}^{K} E\left| p_{1k} - p_{2k} \right| \tag{3}$$

$$\xi_1^2 = Var\varphi_1(\mathbf{X}_1) \tag{4}$$

where $\mathbf{X}_1 = (p_{11}, \ldots, p_{1K})'$ and $\varphi_1(\mathbf{X}_1) = E\{1 - 0.5 \sum_{k=1}^{K} \left| p_{1k} - p_{2k} \right| \mid \mathbf{X}_1\}$; as long as $\xi_1^2 > 0$, the *U*-statistic is non-degenerate. In our case, given $n_{i\bullet}$ (the total number of food items of the *i*-th individual), and taking the distribution of the food items $\mathbf{X}_i = (x_{i1}, \ldots, x_{iK})'$ to be $M(n_{i\bullet}, \pi_1, \ldots, \pi_K)$, we can then write

$$\varphi_1(\mathbf{X}_1) = 1 - 0.5 \sum_{v_2=1}^{+\infty} P(n_{2\bullet} = v_2) \sum_{k=1}^{K} \sum_{x_{2k}=0}^{v_2} \left| p_{1k} - \frac{x_{2k}}{v_2} \right| \binom{v_2}{x_{2k}} \pi_k^{x_{2k}} (1 - \pi_k)^{v_2 - x_{2k}} \tag{5}$$

If, and only if, there is one single resource category, i.e. $\pi_1 = 1$ and $\pi_2 = \cdots = \pi_K = 0$, so that $\varphi_1(\mathbf{X}_1) = 0$ almost surely, will the *U*-statistic be degenerate. This will happen when, and only when, all individuals are specialized on the same single resource. Otherwise, this *U*-statistic will behave in a reasonable fashion and asymptotic normality is attained. Under asymptotic normality, one can employ the standard deviation for building asymptotic intervals and to perform asymptotically powerful tests. Moreover, the variance of $\breve{O}$ can be obtained by Jackknifing the *U*-statistics by the following formula (Sen 1960; Arversen 1969):

$$Var(\breve{O}) = Kn^2(n-1)\binom{n-1}{2}^{-2}\sum_{c=0}^{2}(cn-4)S_c \qquad (6)$$

where $S_c = \sum w_{i_1,i_2} w_{i_3,i_4}$, for any resample $\{i_1, i_2, i_3, i_4\}$ from $\{1, \ldots, n\}$, $c = 0, 1, 2$ being the number of coincident indices, and the sum in S_c being taken for all such quadruples. Note that resampling is performed among the individuals and not among food items for a single individual. This is done to preserve the underlying stochastic dependency structure within individual resource distributions and, therefore, produce a more robust estimate, without the need and the associated shortcomings of assuming some specific dependency setup. The variance of *E* in turn is given by:

$$Var(E) = Var(1-\breve{O}) = (-1)^2 Var(\breve{O}) = Var(\breve{O}) \qquad (7)$$

so that the variance of $\breve{O}$ holds for *E*.

Discussão Geral

No presente estudo, foi demonstrada a presença de variação interindividual em quatro populações de rãs do Cerrado brasileiro e em uma população de vespas-caçadoras de uma área de Mata Atlântica no Sudeste do Brasil. Chama a atenção o fato de o grau de variação interindividual nas populações estudadas ser tão alto quanto aqueles relatados para comunidades temperadas (Bolnick *et al.* 2003), indicando que existe variação interindividual em comunidades tropicais e que essa variação pode ser tão grande quanto a observada em comunidades temperadas depauperadas. Mais importante, esses resultados contrariam a noção vigente até o momento de que esse fenômeno está restrito a comunidades temperadas de baixa diversidade e resulta da expansão dos nichos decorrente de liberação competitiva (Smith & Skúlason 1996). Supondo que exista competição interespecífica nas comunidades aqui estudadas, os resultados apresentados sugerem a existência de *trade-offs* associados ao uso dos recursos alimentares nessas espécies. Além disso, os resultados indicam que esses *trade-offs* são provavelmente mais fortes do que se imaginava, a ponto de gerarem altos graus de especialização individual mesmo em populações cujos nichos são supostamente mais estreitos do que os exemplos da literatura envolvendo liberação competitiva em comunidades depauperadas (Ebenman & Nilsson 1982; Werner & Sherry 1987; Smith 1990; Robinson *et al.* 1993). Uma questão ainda em aberto, que certamente merece atenção, é a identificação da natureza dos *trade-offs* associados ao uso dos recursos nessas populações.

Uma hipótese que não pode ser descartada, no entanto, é que de fato pode existir um tipo de liberação competitiva nas populações estudadas, ainda que em escala sazonal. Essa liberação competitiva poderia ocorrer nos sistemas estudados se a competição interespecífica é alta na estação seca, quando a abundância de recursos é baixa, e, na estação úmida, quando os recursos são abundantes, a competição interespecífica deixa de ser importante e a população passa a incluir novos itens alimentares. Esse é um cenário totalmente compatível com a observação de que, nas espécies de rãs estudadas, os nichos populacionais foram mais amplos na estação úmida, quando a abundância de recursos no Cerrado é maior do que na estação seca (Pinheiro *et al.* 2002; G. Machado, com. pess.). O mesmo padrão de expansão do nicho populacional na estação úmida foi observado na vespa-caçadora estudada, *Trypoxylon albonigrum.* Independentemente dos fatores ecológicos subjacentes à expansão dos nichos nas populações estudadas, os resultados demonstraram que o grau de variação interindividual em populações naturais pode variar em uma escala de tempo muito menor (sazonal) do que a escala evolutiva comumente invocada ao se discutir esse fenômeno (Van Valen 1965; Lister 1976; Roughgarden 1979). Os resultados, portanto, sugerem que a plasticidade comportamental pode ser um fator importante no surgimento e na manutenção de especialização individual em populações naturais.

O aumento do grau de variação interindividual com o aumento das amplitudes dos nichos nas populações estudadas dá suporte à hipótese da variação do nicho (Van Valen 1965). De fato, Bolnick *et al.* (no prelo), analisando as populações de rãs e a população de esgana-gatas aqui estudadas, além de populações de perca-da-Eurásia, *Perca fluviatilis* (Svanbäck & Persson 2004), dos gastrópodes marinhos *Nucella emarginata* (West 1986) e *Thais melones* (West 1988) e do lagarto *Anolis sagrei* (Lister 1976), demonstraram que populações mais generalistas (nichos mais amplos) são de fato mais variáveis (maior grau de variação interindividual). Esses resultados contrariam a extensa literatura refutando a hipótese de Van Valen (1965), com a diferença de que Bolnick *et al.* (no prelo) mediram diretamente a variação no uso dos recursos em vez de usar a morfologia como estimador dessa variação. Os resultados do presente trabalho em conjunto com os resultados de Bolnick *et al.* (no prelo) sugerem uma possível falha na interpretação que os ecólogos têm feito da hipótese da variação do nicho, gerada pela ênfase excessiva na morfologia como medida do grau de variação interindividual no uso de recursos.

Os ganhos em capacidade de detectar padrões ao se olhar diretamente para o uso dos recursos também se fizeram sentir na investigação dos efeitos da competição na população estudada do esgana-gata *G. aculeatus*. Com o uso da medida de agregação C_{ws}, foi possível detectar um padrão inédito de partição de recursos em peixes de lagos temperados, mais sutil do que a dicotomia litoral-coluna d'água. A aplicação desse método em outras populações de *G. aculeatus* e em outras espécies de peixes lacustres é promissora no sentido de revelar a generalidade do padrão aqui descrito e de permitir uma melhor compreensão das forças ecológicas e evolutivas responsáveis pelo surgimento dos polimorfismos de recurso.

Fica evidente que o uso dos índices propostos por Bolnick *et al.* (2002) podem enriquecer em muito a percepção do fenômeno da variação intrapopulacional no uso de recursos. Existem ainda muitas questões não-respondidas acerca desse fenômeno, a começar pela sua incidência em populações naturais e, especialmente, em populações tropicais. Ao documentar a existência de variação interindividual no uso de recursos em rãs e vespas tropicais, o presente estudo contribui para o preenchimento dessa lacuna. Além disso, os resultados aqui apresentados chamam a atenção para o importante papel dos *trade-offs* comportamentais como mecanismo subjacente à variação interindividual no uso de recursos. Mesmo no caso dos esgana-gata, em que essa variação tem uma base morfológica, o que foi evidenciado pelas diferenças morfológicas entre as "microguildas", ela é mediada pelo comportamento de forrageio, que parece responder à intensidade da competição. Finalmente, os modelos de evolução de nichos (Roughgarden 1972), de deslocamento de caráter (Taper & Case 1985) e de especiação (Dieckmann & Doebeli 1999) baseiam-se na suposição de que a competição entre os indivíduos decai continuamente com a distância fenotípica. A presença de "microguildas" na população de *G. aculeatus* indica que a competição intra-específica nessa população tende a ser tudo-ou-nada, não decaindo continuamente. A métrica de agregação C_{ws}, portanto, pode nos dar uma idéia do quão realista é a suposição feita nesses modelos. Essas e outras questões somente poderão ser respondidas aplicando-se os índices propostos por Bolnick *et al.* (2002) e os aqui propostos ao estudo dos padrões de uso de recursos em populações naturais. Espera-se que os resultados apresentados aqui reacendam o interesse pelo estudo do fenômeno da variação intrapopulacional no uso de recursos, que permaneceu negligenciado na literatura ecológica nas últimas décadas (Bolnick *et al.* 2003), e que os métodos propostos aqui possam ampliar a nossa capacidade de realizar essa tarefa.

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Apêndice 1

VarIso1: a PC-compatible program that uses empirical diet and δ^{13}C stable isotope data to generate an expected relationship between diet and isotope variation

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VarIso1

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VarIso1 is a PC-compatible program that uses empirical diet and $\delta^{13}C$ stable isotope data to generate an expected relationship between diet and isotope variation.

Downloading this program will place a single .exe file in the folder you save it to.

Overview of the program:

Double-clicking the icon for the program will open an MS-DOS window, and the program will start. You will go through the following steps (discussed in detail further on in this manual):

1. You will be prompted to enter the number of individuals in your sample.
2. You will be prompted to enter the number of prey categories.
3. You will be prompted to enter the vector of proportions of the population overall diet.
4. You will be prompted to enter the vector of prey isotopic signatures.
5. You will be asked if you want to enter the prey dry masses.
    a. If you answer ‘yes’, you will be prompted to enter the vector of prey dry masses.
6. You will be prompted to enter the number of replicate simulations at a given level of diet variation.

Program details:

The idea underlying our simulations is to generate a high number of simulated populations using a set of parameters that you obtained from your empirical sample. For each simulated population, a standardized index of among-individual diet variation and the isotopic variance will be calculated. With these measures you will be able to establish an expected relationship between among-individual diet variation and isotopic variance, which in turn will allow the conversion of the empirical isotopic variance into a measure of diet variation that can be compared across different populations. Details on how this can be done are given below.

Heading numbers follow the preceding outline of the program.

1) Number of individuals

Here you should enter the number of individuals in your empirical sample. In the simulations, populations of this size will be generated (see below).

2) Number of prey categories

Here you should enter the number of prey categories identified in your empirical sample. In the simulations, individuals will be allowed to feed on the number of prey categories you enter (see below).

3) Population diet

Here you should enter the population diet vector **p** containing the proportions q_j of the j resources in the population diet, so that $\mathbf{p} = (q_1, q_2, \ldots, q_j)$. Let's take the following hypothetical diet matrix, composed of five individuals and four prey categories as an example:

Individual	Food type A	Food type B	Food type C	Food type D
1	8	7	2	3
2	15	3	0	0
3	0	7	3	8
4	0	1	5	10
5	0	0	4	12

The calculation of q_j is straightforward and can be done by summing up all prey items falling into category *j* (sum all *i* individuals) and then converting it into a proportion by dividing it by the total number of prey items of the total population diet:

$$q_j = \frac{\sum_i n_{ij}}{\sum_i \sum_j n_{ij}}$$

In the example, the population diet proportions would be:

Food type A	Food type B	Food type C	Food type D
0.26	0.20	0.16	0.38

so that **p** = (0.26, 0.20, 0.16, 0.38).

4) Prey isotopes

Here you should enter the vector **i** of the empirically determined prey isotopes, in common delta notation. In the previous example, this could be **i** = (-22.28, -21.67, -13.32, -19.39).

5) Prey dry masses

It is at the user's option to incorporate the empirically determined prey dry masses in the simulations. If this option is chosen, you should enter the vector **m** of prey dry masses in any arbitrary units (e.g. mg). For example, **m** = (2.04, 1.58, 0.52, 0.25) could be the vector of dry masses (mg) in the previous example.

6) Number of replicates

The simulations will generate populations with different degrees of among-individual diet variation. Initially populations with extreme degrees of diet variation are generated and diet variation gradually decreases towards zero during the course of simulations (see below). The user must determine how many replicates of simulated populations must be generated at each level of diet variation. Based on our own experience, 100 replicates at each level of diet variation is an appropriate number. This will generate 5,700 simulated populations, which is a fairly good number to establish the relationship between diet and isotope variation and is not computationally prohibitive.

7) Simulations

7.1) Indices of diet variation

We chose two indices of among-individual diet variation. The first is Roughgarden's (1979) *WIC/TNW*, in which the population's total niche width (*TNW*) is partitioned into a within-individual component (*WIC*) and a between-individual component (*BIC*), so that *TNW* = *WIC* + *BIC* (note that Roughgarden referred to these as within- and between-phenotype components). One can then measure diet variation by calculating the ratio *WIC/TNW*. This index varies from 0 (maximum diet variation) to 1 (no diet variation). While it may be more intuitive to use *BIC/TNW* as a measure of individual specialization because larger values reflect more diet variation, we stick with *WIC/TNW* to follow historical precedent. This index uses the Shannon-Weaver index as an estimate of *TNW* (Roughgarden 1979). As an alternative, the program also calculates a second measure of individual specialization (*IS*) based on distribution-overlap, which assumes the value 1 if there is no diet variation among individuals and tends to 0 as variation increases (Bolnick et al. 2002). Readers are referred to Bolnick et al. (2002) for the formulas of the indices and details on their calculation.

7.2) Generating simulated population

In this section we will briefly discuss how simulated populations are generated. We refer readers to Araújo *et al.* (in press) for details on the simulations.

As previously stated, each simulated population is composed of the empirically observed number of individuals, *N*. Each individual's resource distribution is assigned by a multinomial sample from the empirical population's resource distribution **p**. We can control the level of diet variation among individuals by setting the number of multinomial draws that each individual takes from the population's distribution. Due to the Law of Large Numbers, individuals given few draws have narrower and, as a consequence, more variable diets than when individuals have many draws. Each simulated individual is given *s* random draws (with replacement) from this multinomial probability distribution.

The goal is to use the resulting number of draws (n_{ij}) of each prey type j to represent a long-term diet vector $\mathbf{p}_i$ for the simulated individual. Although we acquire this vector by a sampling process, we use it to represent the vector of individual long-term diet preferences. If an individual is given only a single draw (s = 1), it will persistently specialize on a single type of prey resource, e.g., $\mathbf{p}_i$ = (1.0, 0, 0…0). Since different individuals will draw different prey from the population vector, s = 1 yields the maximum level of among-individual variation. As s increases, individuals' diet vectors $\mathbf{p}_i$ converge towards the population diet vector $\mathbf{p}$ (Law of Large Numbers) and diet variation declines.

After calculating the $\mathbf{p}_i$ vectors, our simulation uses the empirically-obtained prey isotope signatures to calculate each simulated individual's isotope signature:

$$E(\delta_i) = \sum_j p_{ij} \delta_j$$

In case prey dry masses were also entered, our simulation instead uses both the isotope signatures and dry masses to calculate the individuals' isotopic signature:

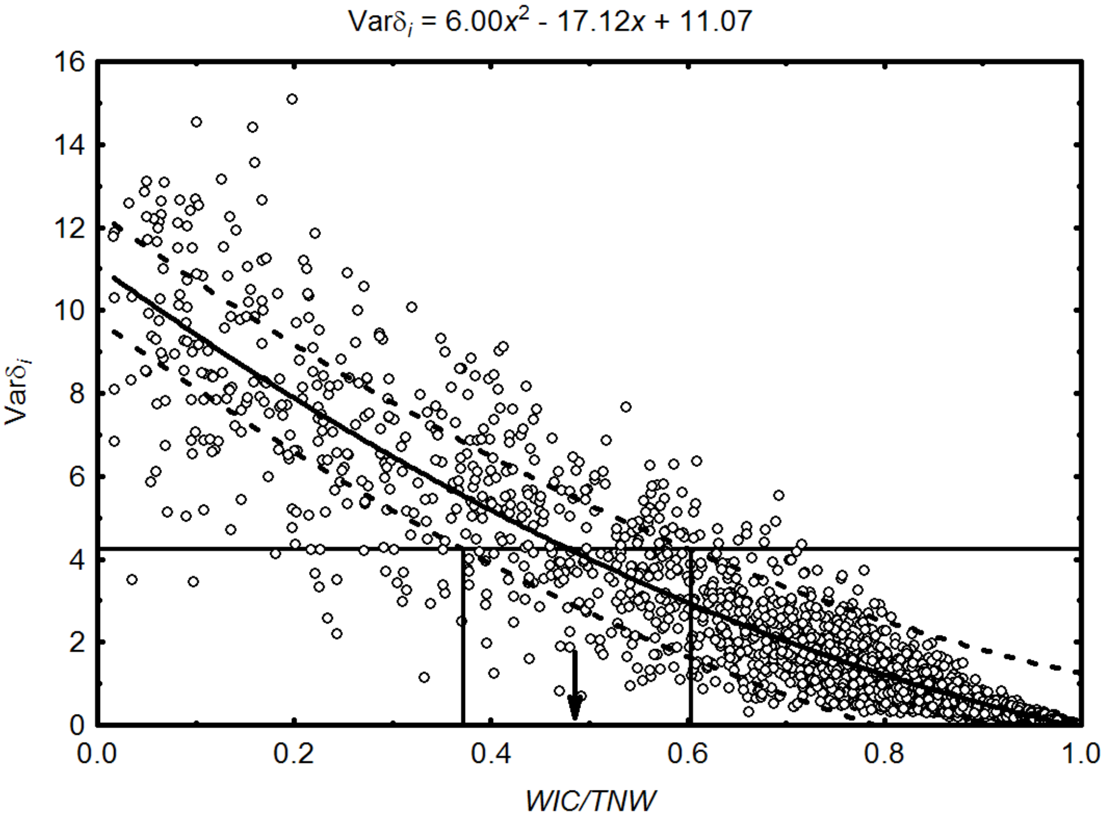
$$E(\delta_i) = \sum_j \frac{p_{ij} m_j}{\sum_j p_{ij} m_j} \delta_j$$

Finally, the program calculates *WIC/TNW, IS*, and the population isotope variance $\mathrm{Var}\,\delta_i$ for the simulated population. The model repeats this procedure for n replicate populations for each of 57 values of s (ranging from 1 to 1,000 in increasing increments).

8) Output

The program outputs a .txt file named VarIso with three columns named 'WIC/TNW', 'IS', and 'Var (isotopes)', in which each line corresponds to one simulated population. This file can be easily imported into Microsoft Excel or common statistics programs to generate scatter plots and regression equations. With this regression equation, one can convert the empirically-estimated isotopic variance into a *WIC/TNW* (or *IS*) value that can be compared with similar measures for other populations. Moreover, regression prediction bands can be estimated and used to establish a confidence interval around the estimated value of *WIC/TNW* (or *IS*). Users can then use the estimated *WIC/TNW* and its confidence interval to check the *WIC/TNW* value estimated from gut contents, as a way of comparing both approaches (see Araújo *et al.*, in press). Both *WIC/TNW* and *IS* can be calculated from gut contents with IndSpec1, a program to calculate indices of individual specialization (Bolnick *et al.* 2002).

To illustrate the use of our method, we provide an example, using the hypothetical sample presented earlier in this manual. Recall that for this sample, **p** = (0.26, 0.20, 0.16, 0.38), **i** = (-22.28, -21.67, -13.32, -19.39), and **m** = (2.04, 1.58, 0.52, 0.25). We also assumed that $N = 30$, and that the empirical Var$\delta_i = 4.25$. We used STATISTICA7.0 to generate the quadratic regression and prediction bands shown below. We used the resulting equation, and the empirical value of Var δ_i, to solve for an estimated value of *WIC/TNW.*



The solid curve indicates the fitted regression; the dashed curves are the prediction bands of the regression; the horizontal solid line indicates the empirically estimated $\text{Var}\,\delta_i = 4.25$; the vertical solid lines define the confidence limits around the expected *WIC/TNW* = 0.48, which is indicated by the arrow. The expected *WIC/TNW* was interpolated from the empirical $\text{Var}\,\delta_i$ using the regression equation.

In case an estimate of the variance in fractionation among individuals (Var_Δ) is available for the organism being studied, this can be used to correct the estimate of the empirical $\text{Var}\,\delta_i$ before interpolating the expected *WIC/TNW*. This can be done by simply subtracting Var_Δ from $\text{Var}\,\delta_i$. See Araújo *et al.* (in press) for a detailed discussion on this topic.

9) Troubleshooting

This program is brand new and has not been extensively tested. If you have trouble, please write to either danbolnick@mail.utexas.edu, or msaraujo@gmail.com, giving us as much detail as possible on the problem.

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Apêndice 2

DIETA1: a PC-compatible program that calculates two indices of intrapopulation variation in resource use, based on complex network theory.

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DIETA1

September 5 2006

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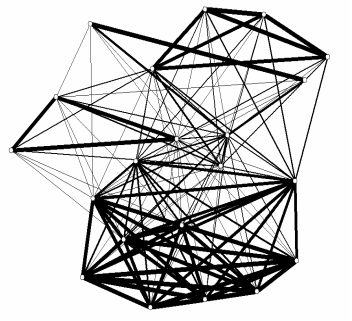

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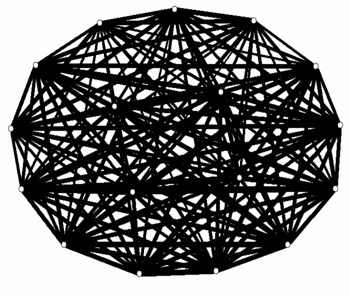

DIETA1 is a PC-compatible program that calculates two indices of intrapopulation variation in resource use, based on complex network theory.

Downloading this program will place a single .exe file in the folder you save it to. It is recommended you create a folder exclusively for DIETA1, save the .exe file to this folder, and place all data text files you want to read in that folder.

Overview of the program:

Double-clicking the icon for the program will open an MS-DOS window, and the program will start. You will go through the following steps (discussed in detail further on in this manual):

1. You will be prompted to enter a data file name. See below for details.
2. You will be asked what type of data is in the file. There are three possible types: (i) proportions; (ii) integers; and (iii) decimal numbers (see below). If the data are not already converted into proportions, the computer will convert the data matrix into a matrix of proportions, calculating the proportion of each individual's diet that falls into a given resource category.
3. If the data are integers, the program goes to step 4, otherwise step 4 is skipped and the program goes directly to step 5.
4. You will be asked if Monte Carlo bootstrap simulations will be run.
    a. In case of a positive answer, the program will need to calculate the proportion of the population diet that falls into each resource category for the simulations. There are two ways of doing this (see below). You will be prompted for which way you prefer. Then you will be asked how many bootstrap replicates (1 to 10,000) you would like to run.
5. You will be asked to enter a value for the 'weight factor' (see below).

6. If Monte Carlo bootstraps will be run, you will be asked if you want to print the file ‘Boot[*filename*].txt’

Program details:

Heading numbers follow the preceding outline of the program.

1) Data entry

DIETA1 reads text files. These can be easily produced in Microsoft Excel by choosing “Save As”, and changing the type option to Text (.txt). The program can also read files with other extension, such as .net, but the data must be in ASCII format and the columns must be separated using <space> or <tab>. The end of the line must be a <new line> character. Save the file into the same folder that holds DIETA1.

1.1) Data file format

The program assumes that the data is in the following format: each row represents the diet data for a given individual; each column represents a distinct class of resource, such as food taxon.

Each cell can be: 1. the proportion in the diet (all entries in the row must sum to 1); 2. counts of individual diet items, as in the following hypothetical example – these will be integers; 3. total mass of each food category in an individual’s gut (floating point numbers will work here). In the following example, the table is of diet item counts for 4 food types on 5 individuals. Each cell is the number n_{ij} of items of resource j that individual i consumed.

Individual	Food type A	Food type B	Food type C	Food type D
1	88	7	2	3
2	152	3	0	0
3	0	7	3	8
4	0	1	5	10
5	0	0	4	12

Important:

1. Do not leave blank spaces where there are no diet items, please fill empty cells with zeros.

2. Do not include the header row naming the resource categories, but **DO** include the first column that identifies individuals. The file for the above data will look like this prior to analysis:

1	88	7	2	3
2	152	3	0	0
3	0	7	3	8
4	0	1	5	10
5	0	0	4	12

2) Data type

The program will prompt you to tell it whether the data is:

1. Already converted into proportions

2. Integers, such as counts of diet items

3. Data with decimal places such as prey mass within each category.

2.1) Converting to proportions

The measures of intrapopulation diet variation rely on mathematical operations on diet proportions, so the first step is transforming the data matrix (**X**) with elements n_{ij} into a matrix of proportions (**P**) with elements p_{ij}.

$$p_{ij} = \frac{n_{ij}}{\sum_j n_{ij}}$$

The matrix for the above data will look like this after the conversion to proportions:

1	0.88	0.07	0.02	0.03
2	0.98	0.02	0.00	0.00
3	0.00	0.39	0.17	0.44
4	0.00	0.06	0.31	0.63
5	0.00	0.00	0.25	0.75

This operation is skipped if the data are already in proportion format. The bootstrapping routine (see below) requires the number of prey items, and cannot be calculated with data already in proportion format or data with decimal places (e.g. prey mass).

4) Data analysis

4.1) Population diet proportions

The Monte Carlo bootstraping routine (see below) depends on the calculation of the population's diet proportions q_j, the proportion of resource j in the population's diet. There are two ways of doing this.

4.1.1) The most straightforward way of calculating q_j is to sum up all prey items falling into category j (sum all i individuals) and then convert it into a proportion by dividing it by the total number of prey items of the total population diet:

$$q_j = \frac{\sum_i n_{ij}}{\sum_i \sum_j n_{ij}}$$

The shortcoming of this approach is that individuals consuming large numbers of prey items will bias the population to look like them. Let's take the above diet matrix as an example. This matrix could represent the food consumption of frogs feeding on terrestrial arthropods. Ants are small, clumped prey that are consumed in large numbers, whereas the other prey categories are large, mobile prey consumed in small numbers:

Individual	Ants	Crickets	Spiders	Beetles
1	88	7	2	3
2	152	3	0	0
3	0	7	3	8
4	0	1	5	10
5	0	0	4	12

The population's proportions would be:

Ants	Crickets	Spiders	Beetles
0.78	0.06	0.05	0.11

which is strongly biased towards ants. In this case, it would maybe be preferable to use prey mass instead of prey number, since there is a correlation between prey size and number in the frogs' diets. Taking the same dataset with dry mass (mg) factored in would yield:

Individual	Ants	Crickets	Spiders	Beetles
1	30.8	58.0	4.1	4.9
2	53.2	24.9	0.0	0.0
3	0.0	58.0	6.1	13.0
4	0.0	8.3	10.2	16.3
5	0.0	0.0	8.2	19.6

and the population's proportions would be:

Ants	Crickets	Spiders	Beetles
0.27	0.47	0.09	0.17

Now, crickets, which are the larger prey consumed would be the most important prey category in the population diet. There is no best way to calculate proportions and the choice between prey number or mass depends on careful considerations on the features of the system being studied, such as the presence of prey number × prey mass correlations. However, bootstrap-based hypothesis testing is restricted to data on the number of prey items, since bootstrapping prey mass data is substantially more complex.

4.1.2) An alternative approach to calculating the population's proportions is to average across each individual's proportions:

$$q_j = \frac{1}{N}\sum_i p_{ij}$$

This will be equivalent to the previous measure when all individuals consume the same number or mass of items. The advantage of this approach is that it weights all individuals equally. Whereas the previous approach to population diet proportions is a measure of resource utilization, this second measure is more a measure of electivity: the proportion of decision-making events that resulted in capturing resource type *j*.

4.2) Calculating the measures of intrapopulation diet variation

We refer readers to Araújo *et al.* (in review) for details on the two proposed indices. Below we give a brief account on them.

4.2.1) The *E* measure of interindividual variation

First, we define *O*, a measure of the network overall degree of pairwise overlap:

$$O = \sum w_{ij} \qquad (1)$$

where

$$w_{ij} = 1 - 0.5\sum_{k=1}^{K} \left| p_{ik} - p_{jk} \right| \qquad (2)$$

is a measure of niche pairwise overlap between individuals *i* and *j* (adapted from Schoener 1968); p_{ik} is the frequency of category *k* in individual *i*'s diet, and p_{jk} is the frequency of category *k* in individual *j*'s diet. The pairwise niche overlap ranges from close to 0 (very little overlap) to 1 (total overlap).

Our measure of the degree of interindividual niche variation in the network is defined as:

$$\breve{O} = \frac{O}{n(n-1)/2} = 2O / n(n-1), \qquad (3)$$

in which n is the number of nodes in the network. $\breve{O}$ will be 1 if there is no interindividual niche variation and will tend to 0 as variation increases. Our measure $\breve{O}$ is is not intuitive, however, as one tends to think that an index to measure interindividual variation will increase its value with increasing interindividual variation and decrease its value otherwise. Therefore, we go a step further and define an index of individual specialization, *E*, as:

$$E = 1 - \breve{O} \qquad (4)$$

Now, in the absence of interindividual niche variation, E will be zero, and will increase towards 1 with the increase of interindividual variation.

4.2.1.1) Variance

A Jackknife estimation of the variance of *E* can be derived using the formalism of *U*-statistics (Arversen 1969). The variance in turn can be used to compare two populations as follows. Given two populations A and B with E_A and E_B measures of interindividual variation, it follows that E_A is aproximately Normal with mean θ_A and variance $\sigma^2{}_A$, i.e. $E_A \sim N\,(\theta_A, \sigma^2{}_A)$ and $E_B \sim N\,(\theta_B, \sigma^2{}_B)$. Therefore, $(E_A - E_B) \sim N\,(\theta, \sigma^2{}_A + \sigma^2{}_B)$. Now, if $\theta = \theta_A - \theta_B$ we want to test the null hypothesis H_0: $\theta = 0$ vs H_1: $\theta \neq 0$. One can perform a simple test using the Normal distribution by calculating

$$\frac{|E_A - E_B|}{\sqrt{\hat{\sigma}_A^2 + \hat{\sigma}_B^2}} \qquad (5)$$

and looking up a Normal distribution table for the *P*-value. If the *P*-value is smaller than α (usually 0.05), one rejects H_0; otherwise there is not enough statistical evidence against the null hypothesis.

4.2.2) The C_{ws} measure of clustering

We propose a measure of the relative degree of clustering in the niche overlap network:

$$C_{ws} = \frac{(C_w - \breve{O})}{(C_w + \breve{O})} \qquad (6)$$

where C_w is the network weighted clustering coefficient.

In a totally random network (in our case, a network consisting of individuals that sample randomly from the population niche), $C_{ws} \sim 0$, indicating no clustering. If individuals form discrete groups specialized on distinct sets of resources, $C_w > \breve{O}$, $0 < C_{ws} \leq 1$, and the network is clustered. The closer C_{ws} is to 1, the higher the degree of clustering. If $C_w < \breve{O}$, $-1 \leq C_{ws} < 0$, the network degree of clustering is actually lower than what would be expected solely on the overall network density of connections, indicating that the individuals' diets are overdispersed. The closer C_{ws} is to -1, the more overdispersed are the individuals' diets.

4.2.3) Visualizing networks

DIETA1 outputs a matrix with all w_{ij} values, and a binary (zeros and ones) matrix in which ones represent edges whose weights are higher then the average network weight $\breve{O}$ (strong edges). Both matrices can be used to visualize networks, and the binary matrix can be used to identify *w-cliques*, which are defined as groups of nodes in which all nodes are connected to each other by the so-defined strong connections (Araújo *et al.*, in review). These *w-cliques* in turn can be used as a way to visualize clusters in the niche overlap network and determine the affiliation of individuals to the different clusters. Another approach that can be taken following the identification of clusters is to map resources onto those clusters. This allows the identification of the resources associated to each cluster, which can be a useful tool in identifying the resources underlying the resource polymorphism.

The visualization of the networks can be done in commonly used softwares of network analyses (e.g. Pajek). The matrices generated by DIETA1 can be imported into Pajek, one of the most popular of such programs (Batagelj & Mrvar 1998) that can be downloaded for free at http://vlado.fmf.uni-lj.si/pub/networks/pajek. Users interested in visualizing their networks are encouraged to read carefully the Pajek manual to get details on how to import the files generated by DIETA1 and use them to do the above-mentioned analyses.

4.2.4) Bootstraping

In case the data are integers, you will be asked if Monte Carlo bootstrap simulations will be run to test the null hypothesis that any observed diet variation arose from individuals sampling stochastically from a shared distribution. In the simulations, each individual is assigned a number of prey items equal to the number of items it was observed eating, and then prey items are randomly assigned to the individual's diet via multinomial sampling from the observed population resource distribution. Next, both *E* and C_{ws} are recalculated for the resulting simulated population. The program can generate up to 10,000 such populations. In the case of *E*, the null hypothesis can be rejected if the empirical value is higher than 95% of the null *E* values. In the case of C_{ws}, which assumes both positive and negative values, the hypothesis test is two-tailed, so that the null hypothesis of $C_w \sim \breve{O}$ can be rejected if C_{ws} is higher than 97.5% of the null C_{ws} values or lower than 97.5% of the null C_{ws} values. This Monte Carlo procedure assumes that every prey item observed in an individual's diet represents an independent feeding event. We acknowledge, however, that this assumption may be violated for prey that are found in tightly clumped groups.

5) The 'weight factor'

The output matrix containing the all the w_{ij} values or the binary matrix containing zeros and ones can be imported by network-visualization programs, such as Pajek, and used to draw networks. If the matrix of w_{ij} values is used, Pajek will use these values to determine the width of the edges, so that users can have a visual representation of the strength of connections between nodes. Pajek reads real numbers varying from zero to infinite, working well with values between 3 and 10. If the raw w_{ij} values ranging from 0 to 1 are used, differences in the edge widths are too subtle to be visualized. The function of the weight factor, which multiplies all w_{ij} values in the matrix, is to circumvent this problem, by scaling the w_{ij} values to values more tractable to Pajek. You will be prompted to enter a weight factor varying from 1 to 100; values between 5 and 10 will generally allow a proper network visualization.

6) Output

The program will output five files as follows (*filename* corresponds to the name of the data file entered by the user):

1) one text file named 'Indices[*filename*].txt' containing the empirical *E* and its variance, var(*E*), as well as the empirical C_{ws};

2) one optional text file named '*P*-values[*filename*].txt' containing the non-parametric *P*-values of *E* and C_{ws} generated by the Monte Carlo bootstrap procedure;

3) one optional text file named 'Boot[*filename*].txt' containing the calculated values of $\breve{O}$, *E*, and C_w for every bootstrap simulation;

4) one *.mat file that can be imported into programs of network analysis named '*filename*.mat' containing the matrix of w_{ij} values multiplied by the 'weight factor' previously entered by the user; *.mat files can be easily opened in Microsoft Notepad; the file for the above hypothetical example will look like this:

```
*Vertices    5
   1 "1"
   2 "2"
   3 "3"
   4 "4"
   5 "5"
*Matrix
      0.000     0.899     0.120     0.113     0.050
      0.899     0.000     0.019     0.019     0.000
      0.120     0.019     0.000     0.674     0.611
      0.113     0.019     0.674     0.000     0.875
      0.050     0.000     0.611     0.875     0.000
```

where the number 5 indicates the number of vertices (nodes) in the network; the numbers below ‘*Vertices’ index the vertices and the characters inside quotes (e.g. “1”) are the labels identifying individuals in the first column of the data file; ‘*Matrix’ indicates the square matrix with *i* rows and *j* columns of the pairwise niche overlaps between individuals *i* and *j* (w_{ij}) multiplied by the ‘weight factor’ entered by the user; in the above example, ‘weight factor’ = 1.0; diagonals are arbitrarily set to zeroes, because pairwise overlaps are only calculated between different individuals.

5) and finally one *.mat file named ‘B[*filename*].mat’ representing the binary matrix of strong edges, in which cells correspond to either 1 (strong edge present) or zero (strong edge absent). The binary matrix for the above example will look like this:

```
*Vertices    5
   1 "1"
   2 "2"
   3 "3"
   4 "4"
   5 "5"
*Matrix
     0    1    0    0    0
     1    0    0    0    0
     0    0    0    1    1
     0    0    1    0    1
     0    0    1    1    0
```

Note that in the binary network only the connections with $w_{ij} > \breve{O}$ (0.338 in the example) are maintained.

7) Troubleshooting

This program is brand new and has not been extensively tested. If you have trouble, please write to either prguima.pm@gmail.com, prguima@gmail.com, or msaraujo@gmail.com, giving us as much detail as possible on the problem. If you wish, you might also send the data files so that we can run the program on them and a have a better idea of where the problem is.

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