Journal of Evolutionary Biology
VOLUME 22 ISSUE 11 NOVEMBER 2009

Contents

Research Papers

2131 Connecting behaviour and performance: the evolution of biting behaviour and bite performance in bats
S. E. Santana and E. R. Dumont
2146 From kissing to belly stridulation: comparative analysis reveals surprising diversity, rapid evolution, and much homoplasy in the mating behaviour of 27 species of sepsid flies (Diptera: Sepsidae)
N. Puniamoorthy, M. R. B. Ismail, D. S. H. Tan and R. Meier
2157 Evidence for autotetraploidy associated with reproductive isolation in *Saccharomyces cerevisiae*: towards a new domesticated species
W. Albertin, P. Marullo, M. Aigle, A. Bourgais, M. Bely, C. Dillmann, D. De Vienne and D. Sicard
2171 Long distance dispersal and the fate of a gene from the colonization front
J. Fayard, E. K. Klein and F. Lefèvre
2183 *Drosophila melanogaster* males respond differently at the behavioural and genome-wide levels to *Drosophila melanogaster* and *Drosophila simulans* females
L. L. Ellis and G. E. Carney
2192 Cooperation as a volunteer's dilemma and the strategy of conflict in public goods games
M. Archetti
2201 Influence of learning on range expansion and adaptation to novel habitats
M. Sutter and T. J. Kawecki
2215 Sperm competition and brain size evolution in mammals
J.-F. Lemaître, S. A. Ramm, R. A. Barton and P. Stockley
2222 Parent–offspring conflict and selection on egg size in turtles
F. J. Janzen and D. A. Warner
2231 Shared and unique features of morphological differentiation between predator regimes in *Gambusia caymanensis*
R. B. Langerhans and A. M. Makowicz
2243 Strong morphological support for the molecular evolutionary tree of placental mammals
M. S. Y. Lee and A. B. Camens
2258 Reproductive barriers between two sympatric beetle species specialized on different host plants
H.-J. Xue, S. Magalhães, W.-Z. Li and X.-K. Yang
2267 The interaction of multiple environmental stressors affects adaptation to a novel habitat in the natterjack toad *Bufo calamita*
B. Rogell, M. Hofman, M. Eklund, A. Laurila and J. Höglund
2278 Allometry and performance: the evolution of skull form and function in felids
G. J. Slater and B. Van Valkenburgh
2288 Local adaptation of *Ruellia nudiflora* (Acanthaceae) to biotic counterparts: complex scenarios revealed when two herbivore guilds are considered
I. Ortegón-Campos, V. Parra-Tabla, L. Abdala-Roberts and C. M. Herrera
2298 Natural and sexual selection against immigrants maintains differentiation among micro-allopatric populations
M. Tobler, R. Riesch, C. M. Tobler, T. Schulz-Mirbach and M. Plath
2305 Intergenomic epistasis causes asynchronous hatch times in whitefish hybrids, but only when parental ecotypes differ
P. J. Woods, R. Müller and O. Seehausen
2320 Inbreeding depression in an asexual population of *Mimulus guttatus*
T. N. Marriage and J. K. Kelly

Short Communications

2232 Space, sympatry and speciation
J. Mallet, A. Meyer, P. Nosil and J. L. Feder
2342 Pattern, process and geographic modes of speciation
B. M. Fitzpatrick, J. A. Fordyce and S. Gavrilets
2348 Synergistic and antagonistic interaction between different branches of the immune system is related to melanin-based coloration in nestling tawny owls
J. Gasparini, R. Piault, P. Bize and A. Roulin
2354 Variable selection patterns on the labellum shape of *Geoblasta pennicillata*, a sexually deceptive orchid
S. Benitez-Vieyra, A. M. Medina and A. A. Cocucci

Cover photo: The pygmy fruit-eating bat *Artibeus phaeotis*, Isla Colon, Panama. Santana & Dumont (pp. 2131–2145) investigated the evolution of behavioural and performance plasticity in 20 species of Neotropical leaf-nosed bats. They found that frugivorous bats like *A. phaeotis* have evolved a higher plasticity in their biting behaviour that allows them to maximize their bite force when feeding on hard fruits. Photo: Sharlene E. Santana.

WILEY-BLACKWELL

This journal is available online at Wiley InterScience.
Visit **www3.interscience.wiley.com** to search the articles
and register for table of contents and e-mail alerts.

Information on this journal can be accessed at:
www.blackwellpublishing.com/jeb

Published for the European Society for Evolutionary Biology (ESEB)
by Wiley-Blackwell

Journal of Evolutionary Biology VOLUME 22 ISSUE 11 NOVEMBER 2009 PAGES 2131–2362 **WILEY-BLACKWELL**

ISSN 1010-061X

JOURNAL OF
Evolutionary Biology

VOLUME 22 ISSUE 11 NOVEMBER 2009

WILEY-BLACKWELL

eseb
European Society for Evolutionary Biology

doi:10.1111/j.1420-9101.2009.01827.x

Connecting behaviour and performance: the evolution of biting behaviour and bite performance in bats

S. E. SANTANA* & E. R. DUMONT†

*Organismic and Evolutionary Biology, University of Massachusetts Amherst, Amherst, MA, USA
†Department of Biology, University of Massachusetts Amherst, Amherst, MA, USA

Keywords:

ancestral states;
behaviour;
bite force;
ecomorphology;
Phyllostomidae;
plasticity.

Abstract

Variation in behaviour, performance and ecology are traditionally associated with variation in morphology. A neglected part of this ecomorphological paradigm is the interaction between behaviour and performance, the ability to carry out tasks that impact fitness. Here we investigate the relationship between biting behaviour and performance (bite force) among 20 species of ecologically diverse bats. We studied the patterns of evolution of plasticity in biting behaviour and bite force, and reconstructed ancestral states for behaviour and its plasticity. Both behavioural and performance plasticity exhibited accelerating evolution over time, and periods of rapid evolution coincided with major dietary shifts from insect-feeding to plant-feeding. We found a significant, positive correlation between behavioural plasticity and bite force. Bats modulated their performance by changing their biting behaviour to maximize bite force when feeding on hard foods. The ancestor of phyllostomids was likely a generalist characterized by high behavioural plasticity, a condition that also evolved in specialized frugivores and potentially contributed to their diversification.

Introduction

The classic paradigm of ecological morphology states that variation in morphology is associated with variation in behaviour and performance, which in turn are subject to selection by environmental demands (Arnold, 1983, 1992; Greene, 1986; Coddington, 1988; Feder & Watt, 1992; Garland & Losos, 1994; Wainwright, 1994; Calsbeek *et al.*, 2007; Gomes & Monteiro, 2008). In this context, performance is defined as a measure of an animal's ability to carry out specific tasks associated with fitness (Huey & Stevenson, 1979; Wainwright, 1994), such as escaping predators (e.g. running speed) and processing prey (e.g. foraging efficiency). Studies that link morphology, performance, and ecological diversity are abundant and have focused on functions relevant to fitness, such as locomotion, predator avoidance, and feeding (e.g. Losos, 1990; Sinervo & Losos, 1991; Huckins, 1997; Aerts *et al.*, 2000; Swartz *et al.*, 2003; Dangles *et al.*, 2005; Herrel *et al.*, 2005; Calsbeek *et al.*, 2007).

In contrast to the substantial progress made in addressing the relationship between morphology, performance and ecology, less is known about the relationship between performance and behaviour, which can be defined as 'what an animal does when faced with behavioural options' (Garland & Losos, 1994). The interaction between behaviour and performance might also be of key importance in the origin of both intra- and inter-specific diversity (Lande, 1981; Brodie, 1993; Jones *et al.*, 2003; Roff & Fairbairn, 2007). The pivotal role of behaviour in the ecomorphological paradigm has long been recognized (Garland & Losos, 1994). While there are often clear links between morphological variation and performance, it can be difficult to assess the role of behaviour in shaping the link between performance and ecology. This is because behaviours are often complex and their components can blend into one another and therefore can be difficult to categorize and quantify. Where behaviour has been adequately quantified, studies of lizard locomotion (e.g. Irschick, 2002; Vanhooydonck & Van Damme, 2003; Schulte *et al.*, 2004; Irschick *et al.*, 2005a), prey capture in actinopterygians (Higham, 2007), and suction-feeding in elasmobranchs (Wilga *et al.*, 2007) have demonstrated significant

Correspondence: Sharlene E. Santana, Organismic and Evolutionary Biology, University of Massachusetts Amherst, Amherst, MA 01003, USA.
Tel.: +1 413 545 3565; fax: 413 545 3243;
e-mail: ssantana@bio.umass.edu

relationships among morphological, performance, behavioural, and ecological traits. For example, elasmobranchs specializing in suction-feeding can enhance their suction performance by increasing their proximity to a substrate or to prey, as suction-feeding is most effective at short distances (Wilga *et al.*, 2007). The fact that animals can use alternative behaviours in different contexts might obscure the relationship between morphology, performance and ecology (Irschick *et al.*, 2005b). By modifying their behaviour, individuals may be able to enhance their performance in ways that allow broader resource use. In a wider context, the relationship between behaviour and performance is crucial to a full understanding of adaptive processes.

Numerous ecological radiations are marked by specialization of the feeding apparatus (Schluter, 1996; Jones *et al.*, 2005; Joy & Crespi, 2007), because feeding is an organismal function that is strongly linked to fitness. Testing the performance of underlying morphological features provides insights into the link between morphology and fitness (Arnold, 1983). In studies of feeding in vertebrates, bite force is an important performance trait because increasing bite force can broaden the spectrum of available prey (e.g. Herrel *et al.*, 2001, 2002; Aguirre *et al.*, 2003), which in turn may enhance fitness. We know that bite force is strongly correlated with both body size and cranial morphology (e.g. Herrel *et al.*, 2005, 2008; Herrel & O'Reilly, 2006; Anderson *et al.*, 2008). However, we don't know whether or how bite force is modulated through variation in biting behaviour, and whether evolutionary changes in biting behaviour are associated with changes in bite force across species.

An ideal system for studying the interactions between behaviour and performance and their relationship with ecology would be one that displays marked diversity in each trait, and in which there has been strong selection on these traits and their interaction. Neotropical leaf-nosed bats (family Phyllostomidae) fit these criteria. Phyllostomids comprise more than 160 species that have evolved from an insectivorous ancestor (Wetterer *et al.*, 2000) and specialize on food resources that include arthropods, fruit, nectar and pollen, vertebrates, and blood. Presumably, the phyllostomid radiation was shaped by this incredible trophic specialization and especially by the movement from animal to plant resources (Freeman, 2000; Wetterer *et al.*, 2000; Van Cakenberghe *et al.*, 2002; Teeling *et al.*, 2005). In contrast to other vertebrates, selective pressures on the form and function of the feeding apparatus of these and other bats could have been intensified by the high energetic demands imposed by homoeothermy, large brains, lactation, flight, and by their inability to reduce foods with their forelimbs (e.g. Hutcheon *et al.*, 2002; Vandoros & Dumont, 2004; Voigt & Speakman, 2007). Morphological trends in feeding specializations among phyllostomids include shortening of the rostrum in specialized frugivores, lengthening of the rostrum and tongue in nectarivores, and increased complexity of molar structure in insectivores (Dumont, 1997; Wetterer *et al.*, 2000; Van Cakenberghe *et al.*, 2002; Swartz *et al.*, 2003). It is possible that this remarkable diversity of feeding morphologies was associated with selection for behavioural traits that enhance performance and potentially overcome limitations set by morphology, allowing phyllostomid species to efficiently partition food resources.

Little is known about the coevolution of behaviour and performance within phyllostomid bats. However, previous studies suggest that behaviour and performance are potentially important aspects of resource-partitioning among phyllostomid species. Thus far we know that phyllostomids occupying specialized dietary niches (e.g. nectarivory, dedicated frugivory and sanguivory) exhibit bite forces that depart from expectations based on body size (Aguirre *et al.*, 2002). There is also a close association between maximum bite forces produced by phyllostomids and the size and hardness of food items in their diets. Bats that bite harder tend to eat larger and harder prey (Aguirre *et al.*, 2003). Therefore, bite force appears to be a performance trait of potential ecological relevance. Bats, like most other mammals, break down food with their teeth in order to maximize the surface area over which their digestive fluids can act. For mammals, the process of feeding includes griping, initiating cracks, fracturing, and reducing food items through mastication (Lucas *et al.*, 2002). During these stages, the aim of processing solid food is usually to overcome the forces that maintain the food particles together, and this is done through applying bite forces (Lucas *et al.*, 2004).

Both biomechanical models and empirical data demonstrate that bite force in mammals increases as animals bite with progressively posterior teeth (Spencer, 1998; Greaves, 2000, 2002; Dumont & Herrel, 2003). Therefore, behaviours that modify the location of the bite point should have an effect on bite force, and descriptors of feeding behaviour that include variation in bite point can inform us about the link between feeding behaviour and feeding performance. Biting behaviour, defined by the variation in the teeth used during biting, varies significantly among species of frugivorous phyllostomids (Dumont, 1999). Specialized frugivores bite food items primarily with the molar teeth on one side of their mouths, while unspecialized frugivores bite food items primarily with the molar teeth on both sides of their mouths simultaneously. Many of these bats also switch to biting behaviours that should increase bite force when feeding on hard fruits (Dumont, 1999).

Objectives and hypotheses

Our first goal was to test whether plasticity in biting behaviour and bite performance (bite force) were correlated in 20 species of phyllostomids (Fig. 1). Behavioural plasticity was quantified as the change in biting behav-

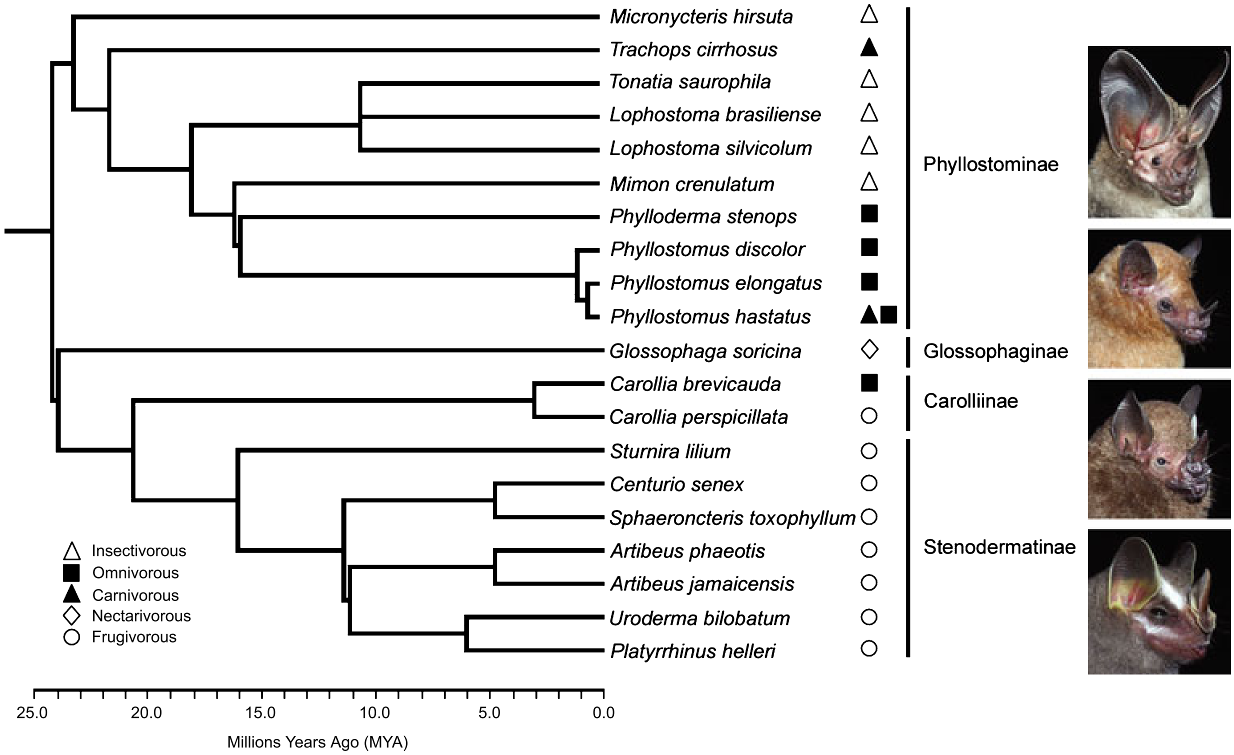

Fig. 1 Phylogenetic relationships and predominant feeding habits among the 20 species of phyllostomids included in this study (tree pruned from Jones *et al.*, 2002, 2005). Representative phyllostomid species pictured, from top to bottom: *Lophostoma silvicolum, Glossophaga soricina, Carollia perspicillata,* and *Uroderma bilobatum.*

iour that animals exhibit when confronted with foods of differing physical characteristics (soft foods vs. hard foods). Specifically, we characterized biting behaviour as the number and types of teeth bats use during biting. We know that different species of bats eat foods with different physical properties (Aguirre *et al.*, 2003; Freeman & Lemen, 2007b), and that individuals modify their biting behaviour according to the physical properties of foods (Dumont, 1999). Therefore, we predicted that biting behaviour will change from soft to hard foods, and this plasticity in biting behaviour will maximize feeding performance on hard foods. Specifically, we predicted that when bats are faced with eating hard foods, they will emphasize biting at the posterior teeth in order to maximize bite force. Across species, we predicted that different species will vary in their tendency to modulate their bite force through behaviour.

To further explore the evolution of biting behaviour and performance, our second goal was to investigate whether large and rapid evolutionary changes in behavioural plasticity occurred in conjunction with periods of major dietary shifts. The null expectation, on the other hand, was that the magnitude and timing of changes in behavioural plasticity occurred randomly across the phylogeny. Theoretically, the evolution of phenotypic plasticity may allow phenotypes to adapt when extreme ecological changes occur (Lande, 2009). These ecological changes would cause an initial drop in fitness, followed by the rapid evolution of plasticity which allows the phenotype to achieve the new optimum. This sequence of events is then followed by slow genetic assimilation of the new phenotype and reduction in plasticity (Lande, 2009). Using ancestral state reconstructions, we tested the hypothesis that the ancestor of phyllostomids was a generalist characterized by biting behaviours similar to those of living insectivorous phyllostomids and with similar, moderate levels of behavioural plasticity. We also investigated whether plasticity levels changed in concert with shifts in diet within the evolution of the lineage. Our analyses of evolutionary changes in biting behaviour and the correlated evolution of biting performance allowed us to explore a relatively neglected aspect of the ecomorphological paradigm.

Methods

Species and study sites

Our analysis included 20 phyllostomid species that span all the major dietary specializations found within the family (Fig. 1, Table 1). We based our dietary classification

Table 1 Predominant feeding habit, sample sizes, body mass, head height and maximum bite force (means ± SE) for the species included in this study.

Species	Diet	*n* feeding behaviour	*n* bite force	Mass (g)	Head height (mm)	Maximum bite force (N)
Micronycteris hirsuta	Insectivore	3	3	14.0 ± 4.51	13.5 ± 0.59	13.1 ± 3.02
Trachops cirrhosus	Carnivore	6	14	28.2 ± 2.47	17.1 ± 0.32	13.5 ± 1.40
Tonatia saurophila	Insectivore	7	9	24.8 ± 2.61	15.8 ± 0.34	16.4 ± 1.74
Lophostoma brasiliense	Insectivore	3	4	9.8 ± 3.91	12.4 ± 0.51	9.4 ± 2.62
Lophostoma silvicolum	Insectivore	4	9	28.1 ± 2.96	15.8 ± 0.39	18.4 ± 1.75
Mimon crenulatum	Insectivore	4	6	14.7 ± 3.19	12.4 ± 0.42	6.8 ± 2.14
Phylloderma stenops	Omnivore	4	4	48.0 ± 3.91	17.6 ± 0.51	13.4 ± 2.62
Phyllostomus discolor	Omnivore	2	3	45.0 ± 5.53	14.5 ± 1.02	9.3 ± 5.24
Phyllostomus elongatus	Omnivore	4	10	33.6 ± 2.77	16.5 ± 0.36	13.0 ± 1.85
Phyllostomus hastatus	Omnivore	7	22	102.7 ± 1.75	22.2 ± 0.22	32.5 ± 1.12
Glossophaga soricina	Nectarivore	4	6	9.3 ± 3.19	9.8 ± 0.42	2.4 ± 2.14
Carollia brevicauda	Omnivore	15	28	16.9 ± 1.48	12.3 ± 0.19	9.2 ± 0.99
Carollia perspicillata	Frugivore	3	63	17.7 ± 0.99	12.8 ± 0.13	8.6 ± 0.66
Sturnira lillium	Frugivore	3	14	16.8 ± 2.26	13.1 ± 0.30	9.2 ± 1.40
Centurio senex	Frugivore	7	9	18.0 ± 1.69	10.6 ± 0.18	10.9 ± 0.85
Sphaeronycteris toxophyllum	Frugivore	1	1	13.0	12.7	3.8
Artibeus phaeotis	Frugivore	3	5	14.4 ± 3.50	11.2 ± 0.46	6.3 ± 2.34
Artibeus jamaicensis	Frugivore	9	196	47.9 ± 0.53	17.8 ± 0.08	18.9 ± 0.37
Uroderma bilobatum	Frugivore	5	15	16.1 ± 2.09	11.9 ± 0.28	5.1 ± 1.40
Platyrrhinus helleri	Frugivore	3	4	14.0 ± 3.91	11.7 ± 0.51	4.8 ± 2.62

on published dietary records for the species (Gardner, 1977; Snow *et al.*, 1980; Ferrarezi & Gimenez, 1996; Aguirre *et al.*, 2003; Giannini & Kalko, 2004; Da Silva *et al.*, 2008). It should be noted that this classification does not necessarily account for all the geographical and temporal variation in natural diets of some of the species (e.g. *Glossophaga soricina*, classified as a nectarivore in this and other studies, may consume fruit and insects in certain seasons and localities; Heithaus *et al.*, 1975; La Val & Fitch, 1977). However, for the purposes of our study, separating bat species into distinctive dietary groups allowed us to test hypotheses concerning the broad relationships between bite performance and biting behaviour within an ecological context. Our results are likely to be robust at a finer level of dietary classification given the clear morphological specializations observed within our broad dietary groups (e.g. Bogdanowicz *et al.*, 1999; Freeman, 2000).

We collected data on phyllostomid species at numerous localities in Venezuela (2006, 2007), Panama (2007), Costa Rica (data published in Dumont, 1999), and Mexico (Dumont *et al.*, in press), where we used mist nets to capture bats in primary or secondary succession forests. Following capture, we determined the bats' age class, sex, and reproductive status. Adult bats were distinguished from sub-adults and juveniles by the degree of ossification in the metacarpal-phalangeal joint of the third finger, which can be detected by palpating and examining the joint against a light (Kunz & Anthony, 1982). Reproductive status in females was determined by direct palpation of the abdomen and nipples. We only used adult males and adult non-pregnant, non-lactating females in this study. All procedures used were approved by the Institutional Animal Care and Use Committee at the University of Massachusetts, Amherst, USA.

Bite performance

Shortly after capture, we measured the bats' bite performance (maximum bite force, in Newtons) using a piezoelectric force transducer (Kistler, type 9203, range ± 500 N, accuracy 0.01–0.1 N; Amherst, NY, USA), attached to a handheld charge amplifier (Kistler, type 5995) and mounted between two bite plates (Herrel *et al.*, 1999b). The tips of the bite plates were covered with medical tape to protect the bats' teeth and to provide a non-skid surface. We adjusted the distance between the bite plates for each individual to accommodate a gape angle of about 30 degrees, because variation in gape angle might affect measurements of bite force (Dumont & Herrel, 2003). Bats were usually eager to bite the transducer, or were stimulated to bite by gentle taps at the sides of their mouth if needed. We recorded at least five bite force measurements for each bat at four bite positions. These bite positions correspond to those observed in phyllostomids feeding on fruits or insects (Dumont, 1999, this paper), and are named after the types and number of teeth involved: shallow bilateral, shallow unilateral, deep bilateral, and deep unilateral. Shallow bites engage the canine and incisor teeth, and deep bites engage the premolar and molar teeth. Unilateral bites use either the left or right tooth row, and bilateral bites use both left and right tooth rows simultaneously. For each individual, the maximum bite force value obtained at each bite type was used to represent that bite type, and the maximum bite force over all bite types was considered the maximum bite force for that individual. Average maximum bite forces were calculated for each species from the individual maximum bite force values, both for each bite position and for overall maximum bite force.

Following bite force recording, we measured body mass (g) using a spring scale, and head dimensions (head length, head width, and head height in millimeters) using digital callipers. Head length was measured as the distance from the tip of the rostrum to the back of the skull; head width was measured at the broadest part of the zygomatic arches; and head height was measured from the highest part of the skull, posterior to the orbit, to the underside of the mandible. Bite force, body mass, and head measurements were log-transformed and bite force was regressed against these size measurements to correct for body size. Log-head height was found to be the best predictor of log-bite force. Residuals of log-bite force (from an Ordinary Least Squares regression) on log-head height were used in subsequent analyses. These statistical analyses were run in SPSS (v. 12.0 for Windows; SPSS Inc., Chicago, IL, USA).

Biting behaviour

Upon completion of performance and morphometric measurements, we placed a subset of bats in individual cloth bags and transported them to a base camp for behavioural observations (Table 1). All other bats were released at their capture sites following the collection of bite force and morphometric data. At the base camp, bats were fed *ad libitum* with fruits or insects until the biting behaviour observations took place. To record biting behaviour, we placed each bat in a small, wire mesh enclosure (40 × 60 × 60 cm) and subsequently offered it food items in a random order. We videotaped the bats while they ate hard and soft food items. For insectivorous bats, hard items consisted of native beetles (Scarabaeidae, length: 13.93 ± 0.21 mm, width: 7.02 ± 0.10 mm) and soft items consisted of native crickets (Tettigonidae, length: 26.44 ± 1.17 mm, width: 6.82 ± 0.47 mm). These insects have been reported to be part of the diet of the insectivorous phyllostomids included in this study (Gardner, 1977; Aguirre *et al.*, 2003). For frugivorous bats, hard and soft foods consisted of spherical pieces (approximately 25 mm in diameter) of apple and banana, respectively. Omnivorous bats were fed both hard and soft fruits and insects. We used non-native fruits for feeding experiments in order to provide foods that would be readily consumed by all species, and to overcome the unavailability of hard and soft native fruits of similar size and shape throughout the length of the study and among localities.

To verify differences in hardness among experimental foods, and to demonstrate that their range of values mimics food hardness encountered in nature, we measured and compared their puncture resistance to that of native foods. To do so, we used a flat-ended needle (1 mm in diameter) attached to the force transducer. There was a significant difference in the hardness (puncture resistance) of soft and hard food items used in the biting behaviour experiments (fruits: $t_{27} = 5.31$, $P < 0.0001$; insects: $t_{233} = 2.56$, $P < 0.05$). Apple pieces were just as hard (5.93 ± 1.78 N) as the hardest fruits naturally consumed by frugivorous phyllostomids, such as *Ficus* ($t_{44} = 0.63$, $P = 0.26$). Conversely, banana pieces were as soft (0.39 ± 0.15 N) as the softest fruits naturally consumed by frugivorous phyllostomids, such as *Piper aduncum* ($t_{19} = 0.30$, $P = 0.38$). The hardness of native insects (soft: 1.49 ± 0.11 N, hard: 2.36 ± 0.07 N) and the rest of foods used in this study fell within the range of bite forces measured in most phyllostomids (Aguirre *et al.*, 2003; Tables 1 and 2).

We recorded the bats as they fed using a digital video camera with night vision (Sony DCR-TRV730 Digital-8 Camcorder). Feeding experiments ended once the bats were satiated. We analyzed the videos of biting behaviour in the lab by classifying the bites used to remove a mouthful (fruits), or to visibly produce physical failure of the exoskeleton (insects). Bite types were classified following methods developed by Dumont (1999), which have been shown to accurately describe differences in biting behaviour among frugivorous phyllostomids. The use of these bite types varies significantly among frugivorous species and across food types. Specialized frugivores predominantly use their molar teeth in an unilateral fashion, while unspecialized frugivores predominantly use their molar teeth in a bilateral fashion (Dumont, 1999). Accordingly, we classified each bite as shallow bilateral, shallow unilateral, deep bilateral, and deep unilateral (described above). We averaged the percentage of each bite type across bats of the same species and used arcsine transformations of these values in our statistical analyses. Unless noted otherwise, only data from hard food feeding experiments were used in comparative analyses of biting behaviour in order to evaluate behaviours that were most likely to be associated with maximum bite force.

Plasticity in behaviour and performance

We investigated behavioural plasticity, i.e. the variation in biting behaviour when switching food types, in two ways. First, we used a three-way analysis of variance (ANOVA) to investigate whether 'food type' (hard vs. soft) had a significant impact on 'bite type' (percentage of shallow bilateral, shallow unilateral, deep bilateral, and deep unilateral bites). In this ANOVA model, the arcsine-transformed percentage of each bite type was the dependent variable, 'food type' and 'bite type' were

Table 2 Bite force measured at each bite type (mean ± SE) and their sample sizes for the species included in this study.

	Maximum bite force at each bite type (N)				
Species	*n*	Shallow bilateral	Shallow unilateral	Deep bilateral	Deep unilateral
Micronycteris hirsuta	3	7.5 ± 1.11	4.9 ± 1.29	12.5 ± 2.08	12.6 ± 1.34
Trachops cirrhosus	10	8.0 ± 0.79	7.9 ± 1.04	11.7 ± 1.41	14.7 ± 1.60
Tonatia saurophila	8	7.3 ± 0.98	4.3 ± 0.93	15.4 ± 1.99	13.9 ± 1.98
Lophostoma brasiliense	3	7.1 ± 0.61	4.7 ± 2.12	8.9 ± 0.35	9.4 ± 0.41
Lophostoma silvicolum	7	12.5 ± 1.77	9.1 ± 1.61	15.1 ± 1.55	19.6 ± 2.48
Mimon crenulatum	6	3.5 ± 0.34	2.4 ± 0.33	5.7 ± 0.27	6.6 ± 0.46
Phylloderma stenops	3	9.7 ± 2.21	6.4 ± 1.39	15.5 ± 3.30	15.5 ± 3.33
Phyllostomus discolor	2	1.6 ± 0.04	0.9 ± 0.02	6.1 ± 0.14	9.5 ± 0.21
Phyllostomus elongatus	6	6.0 ± 1.51	6.4 ± 2.28	11.8 ± 2.30	12.8 ± 2.16
Phyllostomus hastatus	20	17.2 ± 1.21	14.7 ± 1.10	23.2 ± 1.36	32.9 ± 1.95
Glossophaga soricina	6	1.3 ± 0.37	1.1 ± 0.38	1.4 ± 0.29	2.2 ± 0.57
Carollia brevicauda	27	6.2 ± 0.66	5.2 ± 0.58	8.5 ± 0.64	8.7 ± 0.65
Carollia perspicillata	58	5.8 ± 0.32	5.0 ± 0.35	7.9 ± 0.36	8.4 ± 0.37
Sturnira lillium	11	5.7 ± 0.81	5.2 ± 0.77	8.1 ± 1.04	9.2 ± 1.06
Centurio senex	9	8.7 ± 0.68	7.3 ± 0.83	8.7 ± 0.68	10.2 ± 1.14
Sphaeronycteris toxophyllum	1	2.3	1.5	3.8	3.1
Artibeus phaeotis	5	2.8 ± 0.64	1.6 ± 0.38	5.6 ± 0.73	6.3 ± 0.77
Artibeus jamaicensis	149	10.6 ± 0.30	10.5 ± 0.35	14.2 ± 0.35	19.0 ± 0.39
Uroderma bilobatum	13	3.6 ± 0.40	2.7 ± 0.42	4.4 ± 0.44	4.9 ± 0.46
Platyrrhinus helleri	4	2.7 ± 0.60	2.7 ± 0.54	3.5 ± 0.16	4.8 ± 0.69

designated as fixed factors, and ‘species’ was defined as a random factor in order to include variation within species in the construction of error terms. Since all four ‘bite types’ were expressed as percentages and therefore non-independent from each other, we deleted one of the ‘bite type’ categories (shallow unilateral) prior to analyses. This allowed us to test for significance of the factors and generate accurate error terms. Deleting shallow unilateral bites did not affect the results of the ANOVA, as information about this bite type was reflected in the values of the three remaining categories.

Second, we quantified behavioural plasticity by summing the absolute values of the differences in percentages of each bite type when bats fed on soft food vs. hard food. Given that there are four bite types and their percentages add to 100, this value can range from zero (no difference in biting behaviour when feeding on hard vs. soft food items) to 200 (complete shift in biting behaviour when feeding on hard vs. soft food items). This variable, here termed ‘behavioural plasticity’, provided a single value summarizing shifts in biting behaviour for each species that could be used in subsequent phylogenetic analyses.

We investigated performance plasticity by quantifying how changes in biting behaviour translate into changes in maximum biting performance. Admittedly, it is virtually impossible to measure the actual bite forces that are generated during feeding. However, previous comparisons of performance in the field and in the lab support the contention that animals perform at their maximum capacity during challenging situations in nature (Irschick & Losos, 1998; but see Irschick *et al.*, 2005b; Garland, 1999). This lends support to the idea that shifts in feeding behaviour when animals are confronted with challenges, in this case hard food items, signify shifts in performance. To quantify these shifts in performance, we calculated ‘behaviourally adjusted’ bite forces for hard and soft objects. We did this for each species and food type (hard and soft) by multiplying the percentage of each bite type by the maximum bite force measured at that bite position (Table 2), and then summed those products. We removed one outlier, *Phylloderma stenops,* which was two standard deviations below the mean for all species due to its predominant and unique use of shallow bilateral bites during feeding (Fig. 2). We used a paired *t*-test to compare behaviourally adjusted bite forces across all species. To summarize the shift in bite force as animals change from feeding on soft to hard objects, we calculated the difference between the behaviourally adjusted bite forces on the two food types and expressed it as a percentage of each species’ overall maximum bite force. This value is the measure of performance plasticity used in subsequent analyses.

Phylogenetic analyses

We accounted for phylogeny in our analyses by applying a Phylogenetic Generalized Least Squares approach (PGLS, Pagel, 1999) to our measures of behavioural and performance plasticity using BayesTraits (Beta v. 1.1, Pagel & Meade, 2007) and a pruned version of the Jones *et al.* (2002, 2005) species-level supertree of bats (Fig. 1). This supertree estimated the phylogenetic relationships among all 916 extant and nine recently extinct species of

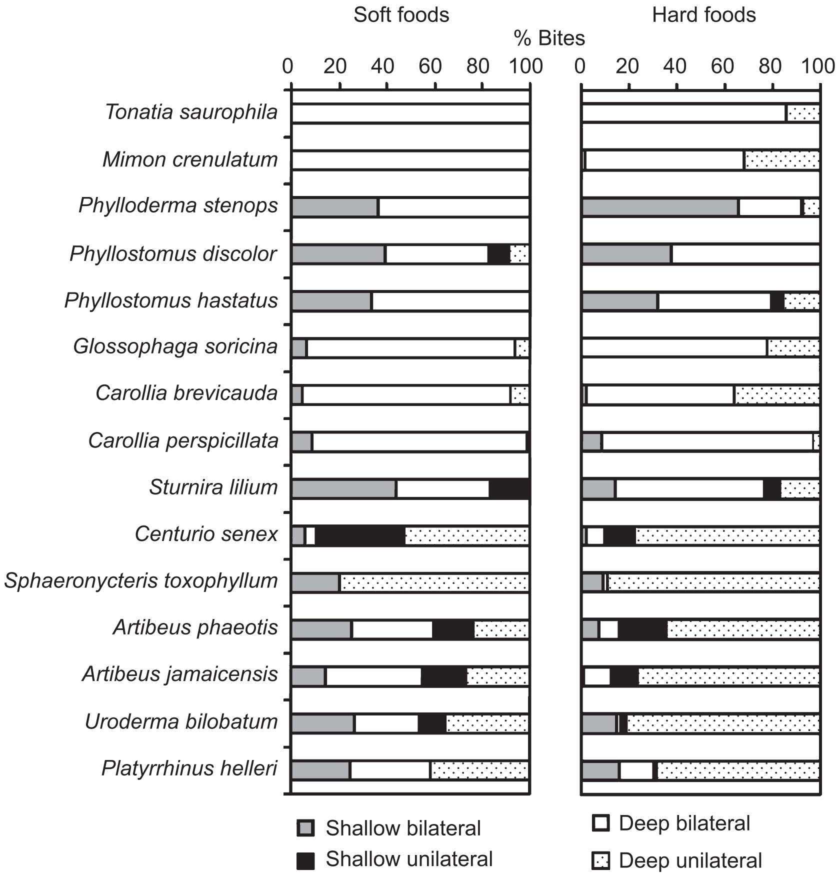

Fig. 2 Proportion of each bite type used by bats while feeding on soft and hard foods. See Fig. 3 for complete data on hard food feeding.

bats (Jones *et al.*, 2002) by combining 105 estimates of bat phylogenetic relationships using Matrix Representation with Parsimony. Dating of the nodes and corresponding branch lengths in the supertree were based on 647 fossil taxa, absolute molecular dates (eight sources), and dates generated from calibrated relative sequence divergences of four mitochondrial and two nuclear genes (Jones *et al.*, 2005). Local molecular clocks, rather than clocklike sequence divergence, were assumed. For the one soft polytomy in the pruned tree (*Lophostoma* and *Tonatia*) we set the corresponding branch lengths to zero using the PDTREE module of Mesquite (v. 2.0, Garland *et al.*, 1993; v. 2.0, Maddison & Maddison, 2007). The Jones *et al.* phylogeny presents plausible estimates of divergence times and phylogenetic relationships among phyllostomid species. However, we acknowledge that some of our results may be sensitive to the accuracy of these estimates (see Shaul & Graur, 2002; Graur & Martin, 2004; Marjanovic & Laurin, 2007) and should be corroborated as additional dated phylogenies become available.

Using the Maximum Likelihood approach in PGLS and a random walk (Brownian motion) model of evolution, we first investigated the patterns of evolution in behaviour and performance plasticity. For each variable, we estimated the maximum likelihood values for three scaling parameters (Pagel, 1999). The first parameter, λ, estimates whether the phylogeny correctly predicts the patterns of variation among species for a given trait, with $\lambda = 0$ indicating phylogenetic independence, and $\lambda = 1$ indicating evolution in accordance with the topology of the phylogeny. The second parameter, δ, estimates whether the rate of trait evolution has accelerated or slowed over time as one moves from the root to the tips of the tree. Values of δ can indicate rapid early evolution at the base of the tree ($\delta < 1$), or accelerating evolution as time progresses ($\delta > 1$). The third parameter, κ, detects apparently higher than expected rates of evolution in braches of different lengths. Values of κ can indicate increased rates of trait evolution in short branches ($\kappa < 1$), or increased rates of trait evolution in long branches ($\kappa > 1$). A value of $\kappa = 0$ denotes extremely rapid evolution over very short time scales. We tested the hypothesis that both behavioural and performance variables follow the same model of evolution. This null model is characterized by evolution following the topology of the phylogeny ($\lambda = 1$) at a gradual pace ($\delta = 1$, $\kappa = 1$).

We tested the significance of the Maximum Likelihood (ML) values of λ, δ, and κ using likelihood ratio tests between the log-likelihoods of a model with the scaling parameter fixed to its hypothetical value (i.e. 1), vs. a model where the parameter took its ML value (Pagel, 1999). These likelihood ratios were compared with a χ^2 distribution with one degree of freedom (Pagel & Meade, 2007). To further verify deviations of the model parameters from the null model, their confidence intervals were calculated using a Markov Chain Monte Carlo and a random walk model of evolution. For tests of correlated evolution among variables, we let λ take its ML value. While internally correcting for phylogenetic dependence using λ, the PGLS model calculates the covariance between the pairs of traits entered into the model. We tested the hypothesis of correlated evolution between behavioural and performance traits by calculating the likelihood ratio between models where correlation was assumed, vs. models where no correlation was assumed.

Finally, we reconstructed ancestral values for the proportions of the four bite types (shallow bilateral, shallow unilateral, deep bilateral, and deep unilateral) during hard object feeding, and behavioural plasticity at each node of the phylogeny in order to investigate whether major shifts in diet were associated with large changes in behaviour. Ancestral reconstruction was done in BayesTraits using a random walk model of evolution and a standard Markov Chain Monte Carlo simulation (Pagel *et al.*, 2004). In this procedure, a Markov chain is constructed from the original data to implement the model of trait evolution. This chain is used to estimate the posterior probability distributions of the values at ancestral nodes in the phylogeny with the associated scaling parameters of interest (λ and δ in our case). We allowed the Markov chain to run for 1 000 000 generations, and set the acceptance rate to range between 20 and 40%. This range is the rate at which changes in the estimated ancestral states are accepted into the chain based on their previous value, therefore controlling the amount of autocorrelation among successive character states of the chain (Pagel, 2008). Convergence of the model was assessed by visual inspection of the parameter values. The posterior probability distributions for the parameter estimates were then used to determine the ancestral character states. The character values presented here correspond to the models with the highest log-likelihoods. We reconstructed the ancestral value of the percentage of each bite type at each node independently. Therefore, the sum of the percentages of the four bite types at any given node did not necessarily add to 100%. To adjust for this discrepancy and make the reconstructed percentages at the ancestral nodes comparable, we recalculated the percentage of each bite type at each node as a proportion of the sum of reconstructed bite type values at that node.

Results

Trait evolution and correlation

We found little difference between the scaling parameters of behavioural and performance plasticity (Table 3). The values of λ implied that evolution in behavioural and performance plasticity was independent of the topology of the phylogeny ($\lambda < 1$, and not significantly different from zero). Maximum likelihood estimates of κ suggested that evolution was significantly more rapid than expected in short branches for both variables. In contrast, the values of δ suggested that the tempo of evolution accelerated over time for behavioural plasticity but not for performance plasticity, in which evolution was nearly gradual.

When comparing feeding behaviour on soft vs. hard foods, we found that phyllostomids significantly change their biting behaviour when they switch from soft to hard foods (Fig. 2, Table 4). Although different species alter their biting behaviour in different ways, the comparison of behaviourally adjusted bite forces demonstrated significant differences between bite forces produced during soft and hard food feeding ($t_{13} = 2.70$, $P < 0.05$). With the possible exception of *Tonatia saurophila*, species exhibited a significant increase in bite forces when switching from soft to hard foods (Table 5). This increase was evident across species despite their broad range in body size (Table 1).

When differences in bite force from soft to hard food feeding were expressed as a percentage of the bat's maximum bite force (i.e. performance plasticity), frugivorous species exhibited the largest change in bite force (Table 5). Performance plasticity was significantly correlated with the plasticity in biting behaviour exhibited by bats when switching from soft to hard foods (LR = 22.837, $\lambda = 0.247$, $r = 0.856$, $P < 0.0001$).

Character history

The reconstruction of ancestral states of bite type proportions (Fig. 3) indicated that the ancestor of all

Table 3 Maximum likelihood (ML) estimates (± 95% confidence intervals) summarizing phylogenetic signal (λ) and the rates of evolution (δ, κ) in the plasticity variables included in this study. *P*-values indicate deviations from tested hypotheses. See methods for description of the variables.

	ML estimates		
Variable	H_0: $\lambda = 1$	H_0: $\delta = 1$	H_0: $\kappa = 1$
Behavioural plasticity	0.56 ± 0.005 ($P < 0.0001$)	2.99 ± 0.016 ($P = 0.0052$)	0 ± 0.016 ($P < 0.0001$)
Performance plasticity	0.90 ± 0.005 ($P = 0.0311$)	2.71 ± 0.015 ($P = 0.1073$)	0.46 ± 0.015 ($P = 0.0409$)

Table 4 Three-way analysis of variance of food type (hard, soft), bite type (shallow bilateral, deep bilateral, deep unilateral), species (listed in Table 1), and their interactions on the percentage of each bite type among 14 species featured in this study.

Source	SS	df	MS	*F*	*P*
Food type	34.795	1	34.795	2.974	0.100
Bite type	16779.563	2	8389.782	4.098	0.039
Species	582.539	12	48.545	0.025	1.000
Food type × bite type	5229.255	2	2614.627	17.136	< 0.0001
Food type × species	110.492	12	9.208	0.060	1.000
Bite type × species	50548.549	24	2106.190	13.646	< 0.0001
Food type × bite type × species	3704.177	24	154.341	1.638	0.280

Table 5 Estimated bite force during soft and hard object feeding, and the difference between these two values as a percentage of each bat's maximum bite force (performance plasticity). Bite forces during soft object feeding are significantly different from those during hard object feeding (paired *t*-test, $t_{13} = 2.70$, $P < 0.05$).

Species	Bite force soft (N)	Bite force hard (N)	Performance plasticity
Tonatia saurophila	15.41	15.19	−1.31
Mimon crenulatum	5.65	5.97	4.70
Phyllostomus discolour	4.08	4.31	2.61
Phyllostomus hastatus	21.22	22.36	3.51
Glossophaga soricina	1.41	1.54	5.84
Carollia brevicauda	8.44	8.55	1.23
Carollia perspicillata	7.70	7.74	0.55
Sturnira lilium	6.55	7.76	13.12
Centurio senex	8.95	9.68	7.17
Sphaeronycteris toxophyllum	2.95	3.05	2.72
Artibeus phaeotis	4.39	5.06	10.67
Artibeus jamaicensis	14.24	17.44	16.92
Uroderma bilobatum	4.82	5.29	8.34
Platyrrhinus helleri	3.83	4.25	8.83

phyllostomids was characterized by the predominant use of deep biting, especially deep bilateral bites. The values of λ associated with the ancestral reconstruction exhibited strong phylogenetic signal for deep bites (parameter ± 95% confidence interval; deep bilateral: 0.95 ± 0.002, deep unilateral: 0.96 ± 0.002; shallow bilateral: 0.36 ± 0.004, shallow unilateral: 0.23 ± 0.004), while the values of δ suggested accelerating evolution in only some types of bites (shallow bilateral: 2.587 ± 0.015, shallow unilateral: 1.73 ± 0.014), and the value of κ suggested that evolution was significantly more rapid than expected in short branches for all variables.

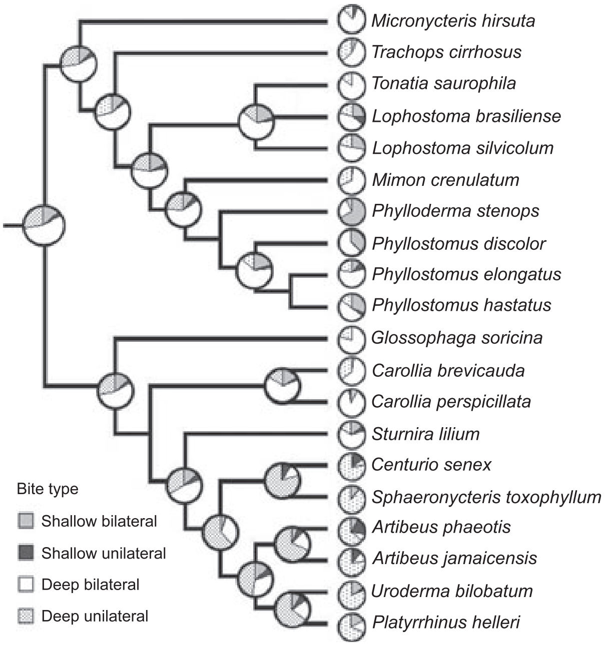

Fig. 3 Ancestral state reconstruction (MCMC method) for proportion of each bite type during hard food feeding. Analyses employ branch lengths shown in Fig. 1. Pie diagrams show character states and their proportion at each node.

From the root node, there was relatively little change in the biting behaviour in the ancestors of the insectivores and omnivores (subfamily Phyllostominae), but a slight increase in deep bilateral bites in the ancestor of the omnivores *Phylloderma* and *Phyllostomus*. Shallow and deep bilateral biting increased in the ancestor of the insectivores *Tonatia* and *Lophostoma*. The clade including the nectarivore and frugivores (subfamilies Glossophaginae, Caroliinae and Stenodermatinae) presented three trends. First, there was no change in the biting behaviour from the root node to the ancestor of the nectarivore *Glossophaga*. Second, there was an increase in deep bilateral biting in the ancestor of unspecialized frugivores (*Carollia* species), mostly at the expense of deep unilateral biting. Finally, there was an increase in deep unilateral biting in the ancestor of the specialized frugivores (Stenodermatinae), a trend that gradually continued towards more terminal nodes.

The reconstruction of the ancestral state for plasticity in biting behaviour (Fig. 4) yielded a relatively high value at the root of the tree (58.34 ± 0.89), when compared to that seen in most of the terminal taxa. The model parameters for the plasticity reconstructions illustrated a strong effect of phylogeny and accelerating evolution (parameter ± 95% confidence interval: $\lambda = 0.70 \pm 0.004$, $\delta = 2.132 \pm 0.014$, $\kappa = 0 \pm 0.015$). Lower plasticity in biting behaviour seems to have evolved early in the two major clades within the phylogeny, the insectivores plus omnivores, and the frugivores. Conversely, higher plasticity seems to have evolved in the ancestor of the specialized frugivores (Stenodermatinae), a condition that reversed to lower plasticity in the ancestor of *Centurio* and *Sphaeronycteris*.

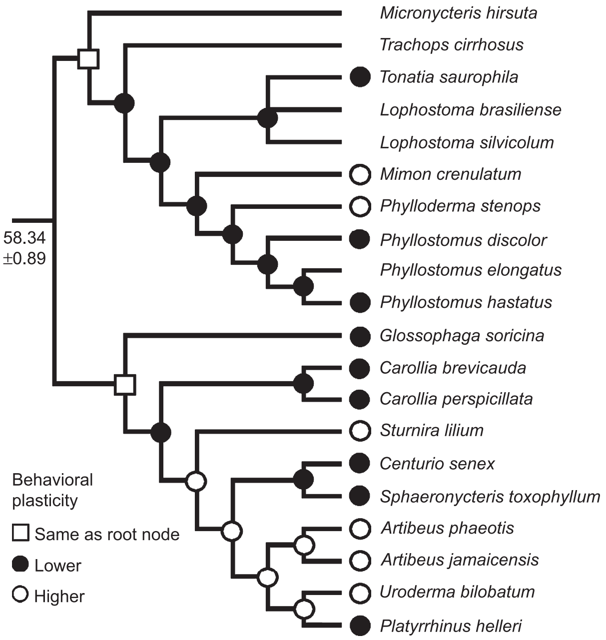

Fig. 4 Ancestral state reconstruction (MCMC method) for plasticity in biting behaviour of bats feeding on soft and hard foods. Analyses employ branch lengths shown in Fig. 1. Nodes marked with squares have values within the root node interval; filled circles are nodes with lower plasticity than the root node interval; empty circles are nodes with higher plasticity than the root node interval; species not marked lack data on the plasticity in biting behaviour.

Discussion

Coevolution of behaviour and performance

The study of the morphology and function of the feeding apparatus has played a central role in our understanding of adaptation because of their contribution to whole-organism fitness. Morphological differences, however, might not always produce measurable differences in performance due to the potentially confounding role of behaviour (Garland & Losos, 1994; Lauder & Reilly, 1996; Irschick & Garland, 2001; Socha & Labarbera, 2005), or the capacity of different morphologies to serve the same function (Wainwright *et al.*, 2005). In the case of phyllostomids, we demonstrate that plasticity in biting behaviour translates into variation in biting performance (i.e. bite force).

We submit that bats modulate bite force by modifying biting behaviours. Although it's possible that bats could apply the same level of force using different biting behaviours, bats do exhibit different bite force maxima during different biting behaviours (Table 2). This strongly suggests that biting performance tracks changes in biting behaviour when bats switch between eating soft and hard foods (Fig. 2). The change in biting behaviour is demonstrated statistically by the significant interaction between food type and bite type in the three-way ANOVA (Table 4). This change has the most extreme implications for performance in frugivores, which can potentially increase their maximum bite forces by more than 10% when eating hard fruits (Table 5). The fact that omnivorous bats modulate biting behaviour in response to food harness (hard vs. soft) rather than food type (insects vs. fruits, data not shown) lends further support to the idea that biting behaviour reflects bite force rather than a response to other differences in the physical properties of fruits and insects. Interestingly, the proportion of each individual bite type is not correlated with size-adjusted bite force during hard food feeding (data not shown). Rather, phyllostomids modulate their bite force by changing the combination of bite types they use in response to changes in the physical characteristics of foods. This observation highlights the importance of investigating complex behaviours (e.g. behavioural plasticity) that relate to whole-organism performance rather focusing narrowly on single aspects of these behavioural events (e.g. proportion of single bite types).

In support of our hypothesis, behavioural and performance plasticity share very similar patterns of evolution: both are largely independent of the underlying phylogeny and exhibit increased rates of evolution on short branches. However, only behavioural plasticity illustrates a significant increase in the rate of evolution over time (i.e. $\delta > 1$). This may be associated with the exceptionally low κ values for this variable, which indicate rapid evolution in short branches. In the phyllostomid phylogeny, short branches occur not only at the base of the tree but also near the tips (Fig. 1). The shortest branches are found at three places in the phylogeny (Fig. 1): (1) the bases of the two clades descending from the root node (Phyllostominae, and Glossophaginae + Carolliinae + Stenodermatinae), (2) the base of the clade including *Phylloderma* and *Phyllostomus*, and (3) the base of the clade formed by *Artibeus*, *Uroderma*, and *Platyrrhinus*. Rapid evolution at these nodes could be associated with changes in diet, namely the occurrence of omnivory in the case of the phyllostomines, and nectarivory and frugivory in other parts of the tree. The switch from animal to plant resources has been associated with the radiation of phyllostomids (Wetterer *et al.*, 2000; Teeling *et al.*, 2005) and could be associated with the evolution of behavioural and performance traits. It will be of particular interest to replicate our analyses using other dated phylogenies to add support to our findings.

Behaviour, performance, and resource use

Bite force appears to constrain the food items a bat can consume in nature. In both fruits and insects eaten by bats, size and hardness are significantly correlated (Aguirre *et al.*, 2003; Dumont, 2003; Freeman & Lemen, 2007a). Working in a tropical bat community, Aguirre *et al.* (2003) demonstrated that food size and hardness play a major role in determining the diets of phyllostomids and other bats. By combining data on bite forces, hardness of native foods, and stomach contents, Aguirre *et al.* were able to demonstrate clear and strong interactions among food hardness, maximum food size, and bite force in insectivorous and omnivorous phyllostomids. Specifically, they found that the maximum size of insects found in stomach contents corresponded to the maximum size of insects that could be punctured given the bat's bite force. Eight of the 11 phyllostomid species investigated by Aguirre *et al.* are included in this study, and most of these consume insects occasionally or regularly. We found that insectivorous bats have a lower tendency to switch biting behaviour (and therefore modulate bite force) when confronted with harder prey, potentially because of their heavy reliance on the molar teeth to crush insects' exoskeletons. Because of this lower behavioural plasticity, we suggest that food hardness, and therefore bite force, may limit dietary breath in these insect-eating bats, excluding large portions of the available dietary spectrum. This is consistent with Aguirre *et al.*'s evidence for a tight association between bite force and body size in insectivorous species.

The relationship between bite force and the size and hardness of foods consumed by frugivorous phyllostomids is less clear. Two extensively studied phyllostomids, *Carollia perspicillata* and *Artibeus jamaicensis*, often feed on fruits whose hardness falls below their maximum bite forces. *Carollia*, a basal frugivore, feeds mostly on very soft fruits such as *Piper aduncum* (e.g. Fleming, 1988; Giannini & Kalko, 2004; Thies & Kalko, 2004). *Artibeus*, a frugivore with a more specialized cranial morphology, concentrates on hard *Ficus* fruits (e.g. Handley & Gardner, 1991; Kalko *et al.*, 1996; Ortega & Castro-Arellano, 2001). *Artibeus* and other specialized frugivores (subfamily Stenodermatinae) vary in the size of fruits they prefer, but these preferences are not clearly associated with the body size, and therefore bite force, of the bats (Kalko *et al.*, 1996; Wendeln *et al.*, 2000). Furthermore, *C. perspicillata* and the nectarivore *Glossophaga soricina* also consume some *Ficus* species (Giannini & Kalko, 2004). It is possible then, that behavioural modifications linked to performance allow frugivorous phyllostomids to mitigate some of the effects of fruit size and hardness and use a broader range of food resources than do insectivorous species. Our results lend support to this idea.

We demonstrate that phyllostomids, especially frugivores, consistently switch their bite types in the direction that would allow them to maximize bite force when confronted with harder foods. This provides compelling evidence that modifications in biting behaviour would allow for an increase in bite performance during challenging situations. Behavioural changes of this sort could allow species to use a broad range of food resources by compensating for differences in performance. Ultimately, physical characteristics of the food items could explain differences in behaviour and could drive the evolution of morphologies adapted to a certain biting style and ecology (e.g. McPeek, 1995; Herrel *et al.*, 1999a, 2002; Vanhooydonck & Van Damme, 2003; Dumont, 2006; Taylor, 2006; Calsbeek *et al.*, 2007; Konuma & Chiba, 2007).

Evolution of biting behaviour and its plasticity

The ancestor of all phyllostomids is thought to have been an insectivore (Ferrarezi & Gimenez, 1996), and our reconstruction of its biting behaviour is consistent with this hypothesis. The ancestral phyllostomid resembled extant insectivores and omnivores in its biting behaviour rather than the more specialized frugivores. Biting behaviours in the ancestors of insectivores and omnivores are relatively unmodified from the root node condition. However, the accelerated evolution of these characters over time appears to have resulted in a remarkable diversity of biting behaviours in the terminal nodes. The predominant use of deep bilateral bites in most phyllostomines could be linked to molar morphologies specialized for breaking down cuticle, cutting meat, and chewing fruit pulp. Associations between molar morphology and insectivory have been demonstrated for other bat taxa (Freeman, 1984, 1988, 1998; Evans, 2005), but this association remains to be tested through comprehensive analyses of phyllostomine dentitions. Within phyllostomines, the cases of *Phylloderma stenops* and *Lophostoma silvicolum* are particularly interesting. *Phylloderma* has uniquely evolved a predominant use of shallow bilateral bites and possesses large, spatulate, interlocking incisors apparently specialized for this function (Freeman, 1992). Due to the lack of dietary information on *Phylloderma*, we can only speculate about the types of prey this bat specializes on. In contrast, we know that male *Lophostoma* use their front teeth to excavate cavities inside hard termite nests (Dechmann *et al.*, 2004, 2009). The ancestral condition for the clade including *Lophostoma* and *Tonatia* has a high proportion of shallow bites with respect to the root node. This biting style may have been co-opted for roost making in *Lophostoma*, as this genus possesses relatively high shallow bilateral bite forces (Dechmann *et al.*, 2009).

In addition to the omnivorous phyllostomines, plant-feeding evolved a second time and became a major dietary habit in the clade including the subfamiles Glossophaginae, Carolliinae and Stenodermatinae (Fig. 1). Unspecialized frugivores (*Carollia*) and the nectarivore (*Glossophaga*) maintain the high proportion of deep bilateral bites of their ancestors despite having switched from animal to plant resources. Bats of these genera tend to include high proportions of insects in their diets (Heithaus *et al.*, 1975; Gardner, 1977; La Val & Fitch, 1977) and may maintain a bite performance and biting behaviour similar to their ancestors for that reason. The specialized frugivores (Stenodermatinae) and their ancestors present an increase in the proportion of deep unilateral bites, a behaviour characteristic of extant species specializing in hard fruits (Dumont, 1999). It seems likely that the consumption of harder and larger fruits is associated with behavioural specialization in the stenodermatines.

In terms of behavioural plasticity, the reconstructed value at the root node is relatively high and the evolution of this character has accelerated over time. This supports our hypothesis that the ancestor of phyllostomids was a generalist in terms of its biting behaviour. A generalized ancestor is commonly assumed in the case of adaptive radiations, but this idea has been evaluated in very few other vertebrates (Schluter, 2000; Salzburger *et al.*, 2005). Lower levels of plasticity have evolved in the ancestors of the phyllostomines, which include most of the omnivores. These bats appear to use a general biting behaviour regardless of the food type, but these behaviours vary among species and have reversed to higher plasticity in two species (*Mimon crenulatum* and *Phylloderma stenops*). Higher values of plasticity evolved in the ancestor of the specialized frugivores (Stenodermatinae), potentially allowing these bats to switch from insects to fruits as a main food source. When behaviours consistently enhance fitness, behavioural plasticity could contribute to adaptation and the diversification of a lineage (Mayr, 1963; West-Eberhard, 1989; Lande, 2009). The specialized frugivore clade includes, in fact, most species of phyllostomids (Simmons, 2004). Nonetheless, when further specialization occurs, such as the case of bats with highly derived morphologies (*Centurio* and *Sphaeronycteris*), plasticity reverts to lower values, perhaps due to either constraints or advantages imposed by morphology. Specifically, the short skulls of these bats could be optimized for resisting torsional loads generated during unilateral biting on hard fruits (Dumont *et al.*, in press), which could constrain the range of biting behaviours these bats can use. However, these same morphological specializations could be advantageous if they allow *Centurio* and *Sphaeronycteris* to access very hard food resources.

Our study provides a comprehensive assessment of the evolution of biting behaviour and performance in a morphologically and ecologically diverse group of vertebrates. We investigate the correlated evolution of plasticity in behaviour and performance, and their potential relationship to feeding ecology. Our findings illustrate coevolution between plasticity in biting behaviour and performance, and the potential association of behaviour with the diversification of fruit-eating lineages. By exploring the links between behavioural and performance traits, we provide the basis for future studies investigating the role of behavioural plasticity in the evolution of performance and perhaps ultimately ecology.

Acknowledgments

We thank Tom Eiting, Jaime Tanner, Jul Davis, the Behaviour and Morphology group at UMass Amherst for numerous and insightful comments during the development of this manuscript. Jesus Molinari facilitated the field seasons in Venezuela, and Johnny Murillo provided help during long hours of field work. This research was supported by NSF IOB 07616 and NSF DBI 0743460 to ERD, a David J. Klingener Endowment Scholarship and a Smithsonian Tropical Research Institute Predoctoral Fellowship to SES. Rodrigo Medellin, Anthony Herrel and Jorge Vargas kindly allowed the use of data from *Centurio senex*.

References

Aerts, P., Van Damme, R., Vanhooydonck, B., Zaaf, A. & Herrel, A. 2000. Lizard locomotion: how morphology meets ecology. *Neth. J. Zool.* **50**: 261–277.

Aguirre, L.F., Herrel, A., Van Damme, R. & Matthysen, E. 2002. Ecomorphological analysis of trophic niche partitioning in a tropical savannah bat community. *Proc. R. Soc B* **269**: 1271–1278.

Aguirre, L.F., Herrel, A., Van Damme, R. & Matthysen, E. 2003. The implications of food hardness for diet in bats. *Funct. Ecol.* **17**: 201–212.

Anderson, R.A., Mcbrayer, L.D. & Herrel, A. 2008. Bite force in vertebrates: opportunities and caveats for use of a nonpareil whole-animal performance measure. *Biol. J. Linn. Soc.* **93**: 709–720.

Arnold, S.J. 1983. Morphology, performance and fitness. *Am. Zool.* **23**: 347–361.

Arnold, S.J. 1992. Constraints on phenotypic evolution. *Am. Nat.* **140**: S85–S107.

Bogdanowicz, W., Fenton, M.B. & Daleszczyk, K. 1999. The relationships between echolocation calls, morphology and diet in insectivorous bats. *J. Zool., Lond.* **247**: 381–393.

Brodie, E.D. 1993. Homogeneity of the genetic variance-covariance matrix for antipredator traits in two natural populations of the garter snake *Thamnophis ordinoides*. *Evolution* **47**: 844–854.

Calsbeek, R., Irschick, D.J. & Pfenning, D. 2007. The quick and the dead: correlational selection on morphology, performance, and habitat use in island lizards. *Evolution* **61**: 2493–2503.

Coddington, J.A. 1988. Cladistic tests of adaptational hypotheses. *Cladistics* **4**: 3–20.

Da Silva, A.G., Gaona, O. & Medellin, R.A. 2008. Diet and trophic structure in a community of fruit-eating bats in Lacandon forest, Mexico. *J. Mammal.* **89**: 43–49.

Dangles, O., Magal, C., Pierre, D., Olivier, A. & Casas, J. 2005. Variation in morphology and performance of predator-sensing system in wild cricket populations. *J. Exp. Biol.* **208**: 461–468.

Dechmann, D.K.N., Kalko, E.K.V. & Kerth, G. 2004. Ecology of an exceptional roost: energetic benefits could explain why the bat *Lophostoma silvicolum* roosts in active termite nests. *Evol. Ecol. Res.* **6**: 1037–1050.

Dechmann, D., Santana, S.E. & Dumont, E.R. 2009. Roost making in bats – adaptations for excavating active termite nests. *J. Mammal.*, in press.

Dumont, E.R. 1997. Cranial shape in fruit, nectar, and exudate feeders: implications for interpreting the fossil record. *Am. J. Phys. Anthropol.* **102**: 187–202.

Dumont, E.R. 1999. The effect of food hardness on feeding behaviour in frugivorous bats (Phyllostomidae): an experimental study. *J. Zool., Lond.* **248**: 219–229.

Dumont, E.R. 2003. Bats and fruit: an ecomorphological approach. In: *Bat Ecology* (T.H. Kunz & M.B. Fenton, eds), pp. 398–429. The University of Chicago Press, Chicago.

Dumont, E.R. 2006. The correlated evolution of cranial morphology and feeding behavior in new world fruit bats. In: *Functional and Evolutionary Ecology of Bats* (A. Zubaid, G.F. McCracken & T.H. Kunz, eds), pp. 160–184. Oxford University Press, USA.

Dumont, E.R. & Herrel, A. 2003. The effects of gape angle and bite point on bite force in bats. *J. Exp. Biol.* **206**: 2117–2123.

Dumont, E.R., Herrel, A., Medellin, R.A., Vargas, J. & Santana, S.E. (in press) Built to bite: cranial design and function in the wrinkle faced bat (*Centurio senex*). *J. Zool.*, doi: 10.1111/j.1469-7998.2009.00618.x.

Evans, A.R. 2005. Connecting morphology, function and tooth wear in microchiropterans. *Biol. J. Linn. Soc.* **85**: 81–96.

Feder, M.E. & Watt, W.B. 1992. Functional biology of adaptation. In: *Genes in Ecology* (R.J. Berry, T.J. Crawford & G.M. Hewitt, eds), pp. 365–392. Cambridge University Press, Cambridge, UK.

Ferrarezi, H. & Gimenez, E.A. 1996. Systematic patterns and the evolution of feeding habits in Chiroptera (Mammalia: Archonta). *J. Comp. Biol.* **1**: 75–95.

Fleming, T.H. 1988. *The Short-Tailed Fruit Bat: a Study in Plant-Animal Interactions*. University of Chicago Press, Chicago.

Freeman, P.W. 1984. Functional cranial analysis of large animalivorous bats (Microchiroptera). *Biol. J. Linn. Soc.* **21**: 387–408.

Freeman, P.W. 1988. Frugivorous and animalivorous bats (Microchiroptera): dental and cranial adaptations. *Biol. J. Linn. Soc.* **33**: 249–272.

Freeman, P.W. 1992. Canine teeth of bats (Microchiroptera) – size, shape and role in crack-propagation. *Biol. J. Linn. Soc.* **45**: 97–115.

Freeman, P.W. 1998. Form, function, and evolution in skulls and teeth of bats. In: *Bat Biology and Conservation* (T.H. Kunz, ed.), pp. 140–156. Smithsonian Institution Press, Washington, DC.

Freeman, P.W. 2000. Macroevolution in Microchiroptera: recoupling morphology and ecology with phylogeny. *Evol. Ecol. Res.* **2**: 317–335.

Freeman, P.W. & Lemen, C. 2007a. An experimental approach to modeling the strength of canine teeth. *J. Zool., Lond.* **271**: 162–169.

Freeman, P.W. & Lemen, C.A. 2007b. Using scissors to quantify hardness of insects: do bats select for size or hardness? *J. Zool., Lond.* **271**: 469–476.

Gardner, A. 1977. Feeding habits. In: *Biology of Bats of the New World Family Phyllostomatidae. Part II* (R.J. Baker, J.K.J. Jones & D.C. Carter, eds), pp. 293–350. Special Publications of the Museum Texas Tech University, Lubbock, Texas.

Garland, T. 1999. Laboratory endurance capacity predicts variation in field locomotor behaviour among lizard species. *Anim. Behav.* **58**: 77–83.

Garland, T. Jr & Losos, J.B. 1994. Ecological morphology of locomotor performance in squamate reptiles. In: *Ecological Morphology: Integrative Organismal Biology* (P.C. Wainwright & S.M. Reilly, eds), pp. 240–302. University of Chicago Press, Chicago.

Garland, T. Jr, Dickerman, A.W., Janis, C.M. & Jones, J.A. 1993. Phylogenetic analysis of covariance by computer simulation. *Syst. Biol.* **42**: 265–292.

Giannini, N.P. & Kalko, E.K.V. 2004. Trophic structure in a large assemblage of phyllostomid bats in Panama. *Oikos* **105**: 209–220.

Gomes, J.L. & Monteiro, L.R. 2008. Morphological divergence patterns among populations of *Poecilia vivipara* (Teleostei: Poeciliidae): test of an ecomorphological paradigm. *Biol. J. Linn. Soc.* **93**: 799–812.

Graur, D. & Martin, W. 2004. Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *Trends Genet.* **20**: 80–86.

Greaves, W.S. 2000. Location of the vector of jaw muscle force in mammals. *J. Morphol.* **243**: 293–299.

Greaves, W.S. 2002. Modeling the distance between the molar tooth rows in mammals. *Can. J. Zoolog.* **80**: 388–393.

Greene, H.W. 1986. Natural history and evolutionary biology. In: *Predator–Prey Relationships: Perspectives and Approaches From the Study of Lower Vertebrates* (M.E. Feder & G.V. Lauder, eds), pp. 99–108. University of Chicago Press, Chicago.

Handley, C.O.J. & Gardner, A.L. 1991. Food habits. In: *Demography and Natural History of the Common Fruit Bat, Artibeus Jamaicenis, on Barro Colorado Island, Panama* (C.O.J. Handley, D.E. Wilson & A.L. Gardner, eds), pp. 141–145. Smithsonian Institution Press, Washington D.C.

Heithaus, E.R., Fleming, T.H. & Opler, P.A. 1975. Foraging patterns and resource utilization in seven species of bats in a seasonal tropical forest. *Ecology* **56**: 841–854.

Herrel, A. & O'Reilly, J.C. 2006. Ontogenetic scaling of bite force in lizards and turtles. *Physiol. Biochem. Zool.* **79**: 31–42.

Herrel, A., Aerts, P., Fret, J. & De Vree, F. 1999a. Morphology of the feeding system in agamid lizards: ecological correlates. *Anat. Rec.* **254**: 496–507.

Herrel, A., Spithoven, L., Van Damme, R. & De Vree, F. 1999b. Sexual dimorphism of head size in *Gallotia galloti*: testing the niche divergence hypothesis by functional analyses. *Funct. Ecol.* **13**: 289–297.

Herrel, A., Van Damme, R., Vanhooydonck, B. & De Vree, F. 2001. The implications of bite performance for diet in two species of lacertid lizards. *Can. J. Zoolog.* **79**: 662–670.

Herrel, A., O'Reilly, J.C. & Richmond, A.M. 2002. Evolution of bite performance in turtles. *J. Evol. Biol.* **15**: 1083–1094.

Herrel, A., Podos, J., Huber, S.K. & Hendry, A.P. 2005. Bite performance and morphology in a population of Darwin's finches: implications for the evolution of beak shape. *Funct. Ecol.* **19**: 43–48.

Herrel, A., De Smet, A., Aguirre, L.F. & Aerts, P. 2008. Morphological and mechanical determinants of bite force in bats: do muscles matter? *J. Exp. Biol.* **211**: 86.

Higham, T.E. 2007. The integration of locomotion and prey capture in vertebrates: morphology, behavior, and performance. *Integr. Comp. Biol.* **47**: 82–95.

Huckins, C.J.F. 1997. Functional linkages among morphology, feeding performance, diet, and competitive ability in molluscivorous sunfish. *Ecology* **78**: 2401–2414.

Huey, R.B. & Stevenson, R.D. 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *Am. Zool.* **19**: 357–366.

Hutcheon, J.M., Kirsch, J.A.W., Garland, T. Jr & Hutcheon, J.M. 2002. A comparative analysis of brain size in relation to foraging ecology and phylogeny in the Chiroptera. *Brain. Behav. Evolut.* **60**: 165–180.

Irschick, D.J. 2002. Evolutionary approaches for studying functional morphology: examples from studies of performance capacity. *Integr. Comp. Biol.* **42**: 278–290.

Irschick, D.J. & Garland, T. Jr 2001. Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. *Annu. Rev. Ecol. Syst.* **32**: 367–396.

Irschick, D.J. & Losos, J.B. 1998. A comparative analysis of the ecological significance of maximal locomotor performance in Caribbean *Anolis* lizards. *Evolution* **52**: 219–226.

Irschick, D.J., Carlisle, E., Elstrott, J., Ramos, M., Buckley, C., Vanhooydonck, B., Meyers, J.A.Y. & Herrel, A. 2005a. A comparison of habitat use, morphology, clinging performance and escape behaviour among two divergent green anole lizard (*Anolis carolinensis*) populations. *Biol. J. Linn. Soc.* **85**: 223–234.

Irschick, D.J., Herrel, A., Vanhooydonck, B., Huyghe, K. & Damme, R. 2005b. Locomotor compensation creates a mismatch between laboratory and field estimates of escape speed in lizards: a cautionary tale for performance-to-fitness studies. *Evolution* **59**: 1579–1587.

Jones, K.E., Purvis, A., Maclarnon, A.N.N., Bininda-Emonds, O.R.P. & Simmons, N.B. 2002. A phylogenetic supertree of the bats (Mammalia: Chiroptera). *Biol. Rev.* **77**: 223–259.

Jones, A.G., Arnold, S.J. & Bürger, R. 2003. Stability of the G-matrix in a population experiencing pleiotropic mutation, stabilizing selection, and genetic drift. *Evolution* **57**: 1747–1760.

Jones, K.E., Bininda-Emonds, O.R.P. & Gittleman, J.L. 2005. Bats, clocks, and rocks: diversification in patterns in Chiroptera. *Evolution* **59**: 2243–2255.

Joy, J.B. & Crespi, B.J. 2007. Adaptive radiation of gall-inducing insects within a single host-plant species. *Evolution* **61**: 784–795.

Kalko, E.K.V., Herre, E.A. & Handley, C.O. 1996. Relation of fig fruit characteristics to fruit-eating bats in the new and old world tropics. *J. Biogeogr.* **23**: 565–576.

Konuma, J. & Chiba, S. 2007. Trade-offs between force and fit: extreme morphologies associated with feeding behavior in carabid beetles. *Am. Nat.* **170**: 90–100.

Kunz, T.H. & Anthony, E.L.P. 1982. Age estimation and post-natal growth in the bat *Myotis lucifugus*. *J. Mammal.* **63**: 23–32.

La Val, R.K. & Fitch, H.S. 1977. Structure, movements and reproduction in three Costa Rican bat communities. *Occ. Pap. Mus. Hist. Univ. Kansas* **69**: 1–27.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *Proc. Natl Acad. Sci. USA.* **78**: 3721–3725.

Lande, R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *J. Evol. Biol.* **22**: 1435–1446.

Lauder, G.V. & Reilly, S.M. 1996. The mechanistic basis of behavioral evolution: comparative analysis of muscoskeletal function. In: *Phylogenies and the Comparative Method in Animal Behavior* (E.P. Martins, ed.), pp. 105–137. Oxford University Press, New York.

Losos, J.B. 1990. Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecol. Monogr.* **60**: 369–388.

Lucas, P.W., Prinz, J.F., Agrawal, K.R. & Bruce, I.C. 2002. Food physics and oral physiology. *Food Qual. Prefer.* **13**: 203–213.

Lucas, P.W., Prinz, J.F., Agrawal, K.R. & Bruce, I.C. 2004. Food texture and its effect on ingestion, mastication and swallowing. *J. Texture Stud.* **35**: 159–170.

Maddison, W.P. & Maddison, D.R. 2007. *Mesquite: a Modular System for Evolutionary Analysis*. Version 2.01. Available at: http://mesquiteproject.org. [Accessed on 13 February 2008].

Marjanovic, D. & Laurin, M. 2007. Fossils, molecules, divergence times, and the origin of lissamphibians. *Syst. Biol.* **56**: 369–388.

Mayr, E. 1963. *Animal Species and Evolution*. Harvard University Press, Cambridge, Massachusetts.

McPeek, M.A. 1995. Morphological evolution mediated by behavior in the damselflies of two communities. *Evolution* **49**: 749–769.

Ortega, J. & Castro-Arellano, I. 2001. *Artibeus jamaicensis*. *Mammalian Species* **662**: 1–9.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. *Nature* **401**: 877–884.

Pagel, M. 2008. *Continuous – Draft Manual*. Available at http://www.evolution.rdg.ac.uk. [Accessed on 13 March 2008].

Pagel, M. & Meade, A. 2007. *Bayestraits, version 1. 1 – Draft Manual*. Available at: http://www.evolution.rdg.ac.uk. [Accessed on 13 March 2008].

Pagel, M., Meade, A. & Barker, D. 2004. Bayesian estimation of ancestral character states on phylogenies. *Syst. Biol.* **53**: 673–684.

Roff, D.A. & Fairbairn, D.J. 2007. The evolution of trade-offs: where are we? *J. Evol. Biol.* **20**: 433.

Salzburger, W., Mack, T., Verheyen, E. & Meyer, A. 2005. Out of Tanganyika: genesis, explosive speciation, key-innovations and phylogeography of the haplochromine cichlid fishes. *BMC Evol. Biol.* **5**: 17.

Schluter, D. 1996. Ecological causes of adaptive radiation. *Am. Nat.* **148**: S40–S64.

Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, New York.

Schulte, J.A., Losos, J.B., Cruz, F.B. & Nunez, H. 2004. The relationship between morphology, escape behaviour and microhabitat occupation in the lizard clade *Liolaemus* (Iguanidae: Tropidurinae: Liolaemini). *J. Evol. Biol.* **17**: 408–420.

Shaul, S. & Graur, D. 2002. Playing chicken (*Gallus gallus*): methodological inconsistencies of molecular divergence date estimates due to secondary calibration points. *Gene* **300**: 59–61.

Simmons, N.B. 2004. Order Chiroptera. In: *Mammal Species of the World: a Taxonomic and Geographic Reference* (D.E. Wilson & D.M. Reeder, eds), pp. 2–340. Smithsonian Institution Press, Washington DC.

Sinervo, B. & Losos, J.B. 1991. Walking the tight rope – arboreal sprint performance among *Sceloporus occidentalis* lizard populations. *Ecology* **72**: 1225–1233.

Snow, J.L., Jones, J.K. Jr & Webster, W.D. 1980. *Centurio senex*. *Mammalian species* **138**: 1–3.

Socha, J.J. & Labarbera, M. 2005. Effects of size and behavior on aerial performance of two species of flying snakes (*Chrysopelea*). *J.Exp. Biol.* **208**: 1835–1847.

Spencer, M.A. 1998. Force production in the primate masticatory system: electromyographic tests of biomechanical hypotheses. *J. Hum. Evol.* **34**: 25–54.

Swartz, S.M., Freeman, P.W. & Stockwell, E.W. 2003. Ecomorphology of bats: comparative and experimental approaches relating structural design to ecology. In: *Bat Ecology* (T.H. Kunz

& M.B. Fenton, eds), pp. 257–300. The University of Chicago Press, Chicago.

Taylor, A.B. 2006. Feeding behavior, diet, and the functional consequences of jaw form in orangutans, with implications for the evolution of *Pongo*. *J. Hum. Evol.* **50**: 377–393.

Teeling, E.C., Springer, M.S., Madsen, O., Bates, P., O'Brien, S.J. & Murphy, W.J. 2005. A molecular phylogeny for bats illuminates biogeography and the fossil record. *Science* **307**: 580–584.

Thies, W. & Kalko, E.K.V. 2004. Phenology of neotropical pepper plants (Piperaceae) and their association with their main dispersers, two short-tailed fruit bats, *Carollia perspicillata* and *C. castanea* (Phyllostomidae). *Oikos* **104**: 362–376.

Van Cakenberghe, V., Herrel, A. & Aguirre, L.F. 2002. Evolutionary relationships between cranial shape and diet in bats (Mammalia: Chiroptera). In: *Topics in Functional and Ecological Vertebrate Morphology* (P. Aerts, K. D'Août, A. Herrel & R. Van Damme, eds), pp. 205–236. Shaker Publishing, Maastricht, The Netherlands.

Vandoros, J.D. & Dumont, E.R. 2004. Use of the wings in manipulative and suspensory behaviors during feeding by frugivorous bats. *J. Exp. Zool. Part A Comp. Exp. Biol.* **301A**: 361–366.

Vanhooydonck, B. & Van Damme, R. 2003. Relationships between locomotor performance, microhabitat use and antipredator behaviour in lacertid lizards. *Funct. Ecol.* **17**: 160–169.

Voigt, C.C. & Speakman, J.R. 2007. Nectar-feeding bats fuel their high metabolism directly with exogenous carbohydrates. *Funct. Ecol.* **21**: 913–921.

Wainwright, P.C. 1994. Functional morphology as a tool in ecological research. In: *Ecological Morphology* (P.C. Wainwright & S.M. Reilly, eds), pp. 42–59. The University of Chicago Press, Chicago.

Wainwright, P.C., Alfaro, M.E., Bolnick, D.I. & Hulsey, C.D. 2005. Many-to-one mapping of form to function: a general principle in organismal design? *Integr. Comp. Biol.* **45**: 256–262.

Wendeln, M.C., Runkle, J.R. & Kalko, E.K.V. 2000. Nutritional values of 14 fig species and bat feeding preferences in Panama. *Biotropica* **32**: 489–501.

West-Eberhard, M.J. 1989. Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* **20**: 249–278.

Wetterer, A.L., Rocman, M.V. & Simmons, N.B. 2000. Phylogeny of phyllostomid bats (Mammalia: Chiroptera): data from diverse morphological systems, sex chromosomes and restriction sites. *B. Am. Mus. Nat. Hist.* **248**: 1–200.

Wilga, C.D., Motta, P.J. & Sanford, C.P. 2007. Evolution and ecology of feeding in elasmobranchs. *Integr. Comp. Biol.* **47**: 55–69.

Received 10 June 2009; revised 20 July 2009; accepted 21 July 2009