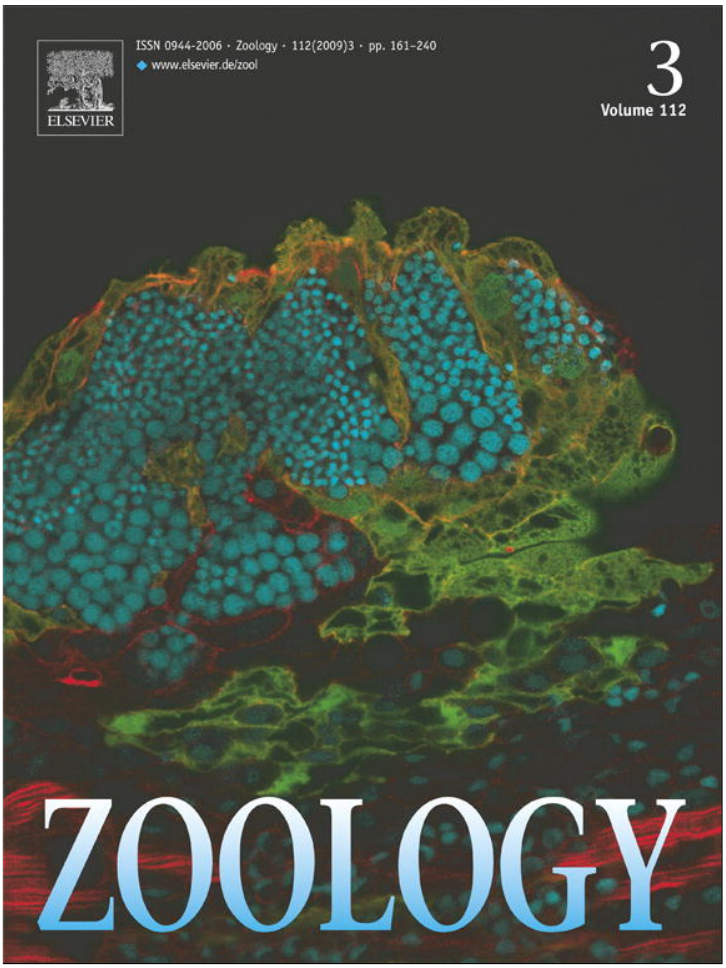
Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright

Author's personal copy

Available online at www.sciencedirect.com
ScienceDirect
Zoology 112 (2009) 217–226
ZOOLOGY
www.elsevier.de/zool

Morphology, ornaments and performance in two chameleon ecomorphs: is the casque bigger than the bite?

G. John Measey[a,b,*], Kevin Hopkins[a], Krystal A. Tolley[a]

[a]*Applied Biodiversity Research, Kirstenbosch Research Centre, South African National Biodiversity Institute, Private Bag X7, Claremont Cape Town 7735, South Africa*
[b]*Department of Biodiversity and Conservation Biology, University of the Western Cape, Private Bag X17, Bellville 7535, South Africa*

Received 12 June 2008; received in revised form 26 August 2008; accepted 13 September 2008

Abstract

The evolution of ecomorphs within a species may represent either unique evolutionary events or multiple convergent events in similar environments. Functional studies of differing morphological traits of ecomorphs have been important to elucidate their role in adaptive radiations. The Cape dwarf chameleon, *Bradypodion pumilum*, has two ecomorphs: a large, brightly colored, ornate form found in closed habitats, and a small, dull form with reduced ornamentation found in open vegetation. The typical form is known to use casque size to communicate fighting ability, but it is unknown whether this is an honest signal and whether casque size is related to bite force. We show through a population genetic analysis that these ecomorphs are not separate genetic lineages but the result of multiple transitions between closed and open habitats. From measurements of ornamental and non-ornamental morphological characters and bite force in 105 chameleons, we find that bite force is significantly related to head size and is best predicted by head width. Bite force was reasonably predicted by casque height in ecomorphs from closed habitats, but not in those from open habitats. For size-adjusted data, open habitat males had wider heads, biting harder than closed habitat males. Our data suggest honesty in signaling for closed habitat ecomorphs, but for open habitat ecomorphs communication is different, a finding commensurate with the common framework for species radiations.
© 2009 Elsevier GmbH. All rights reserved.

Keywords: Adaptive radiation; *Bradypodion pumilum*; Combat; Conspecific signaling; Bite force

Introduction

The proximate cause of adaptive radiation is proposed to be the result of divergent natural selection originating with environments and resource competition (Dobzhansky, 1951; Simpson, 1953; Schluter, 2000). The ecological theory of adaptive radiation states that environments inhabited by different populations can lead to adaptive radiation through phenotypic differentiation (Schluter, 2000). A common framework for species radiations includes three ordered stages where lineages diverge due to differences in habitat use, morphological specialization and finally, communication (Streelman and Danley, 2003). An intermediary stage within this framework is the development of

*Corresponding author at: Applied Biodiversity Research, Kirstenbosch Research Centre, South African National Biodiversity Institute, Private Bag X7, Claremont Cape Town 7735, South Africa.
E-mail address: john@measey.com (G.J. Measey).

0944-2006/$ - see front matter © 2009 Elsevier GmbH. All rights reserved.
doi:10.1016/j.zool.2008.09.005

Author's personal copy

distinct ecomorphs; populations whose appearance is influenced by ecological factors but without taxonomic significance. Ecomorphs need not be unique evolutionary lineages, and evidence from both morphological and genetic studies shows that these can evolve convergently many times in similar environments; documented examples include fish, amphibians, reptiles and mammals (e.g. Wake and Larson, 1987; Kocher et al., 1993; Losos et al., 1998; Fleagle and McGraw, 1999). Studies of the functional significance of morphological traits in these ecomorphs have been pivotal in understanding the evolutionary processes involved in adaptive radiations (e.g. Irschick and Losos, 1998).

Bradypodion pumilum (Daudin) (the Cape dwarf chameleon) has two distinct ecomorphs. Individuals from closed habitats are large, have high casques (parietal crest) and are brightly colored with pink flank patches (see Fig. 1A), which have been implicated in conspecific signaling (Stuart-Fox et al., 2006). In open habitat, chameleons are small, have a reduced casque, are dull in coloration and lack flank color patches (Stuart-Fox and Moussalli, 2007; Stuart-Fox et al., 2007; Tolley and Burger, 2007; see Fig. 1B). The spatial distribution of these ecomorphs is disjunct and dependent upon vegetation type. If gene flow is restricted between vegetation types, the opportunity for genetic differentiation may be present; the result being a single transition between closed and open habitats (i.e. separate lineages). If gene flow is unrestricted, a pattern of isolation by distance should persist. Each ecomorph could represent either multiple states of a phenotypically labile morphotype, or selection acting on the phenotype within each habitat (Tolley et al., 2006). In open habitats, the lack of bright color patches (Stuart-Fox et al., 2007) and reduced ornaments suggests that this ecomorph does not rely on signaling to offset combat (Stuart-Fox and Moussalli, 2008), which would favor maximizing aggressive performance, while reducing ornamentation. In closed habitats, greater investment in signaling could lead to a large head as a signal to conspecifics, rather than maximizing some performance measure.

In chameleons, intra- and inter-sexual combat is made through biting opponents, which can result in severe injuries (Stuart-Fox and Whiting, 2005; Tolley and Burger, 2007; personal observation). Excellent visual acuity allows the assessment of conspecifics from a distance by complex signals, which may help avoid combat; these include behavior, ornamentation and color (Kelso and Verrell, 2002; Stuart-Fox et al., 2006). For *B. pumilum* the outcome of male–male combat can be predicted by the size of the casque and pink patch on the flanks of males (Stuart-Fox et al., 2006). However, it is not known whether ornaments are an honest signal of fighting performance and some signalers may reduce their likelihood of injury by deceiving receivers (Dawkins and Guilford, 1991). As chameleons are known to use biting in conflicts, bite force is an ecologically relevant task (cf. Huey and Stevenson, 1979) and can be used as a measure of fighting performance. Many chameleons have very prominent casques, bony protrusions posterior and superior to the orbits, giving the appearance of a taller head (see Fig. 1A and B). In some lizards, increasing head height is positively correlated with bite force (Herrel et al., 2001), and in chameleons high casques provide muscle attachment points (Rieppel, 1981), possibly increasing bite force and acting as honest signals.

In this study, we use a combination of molecular, morphometric and performance measurements to address a series of hypotheses on the evolution of ecomorphs, signaling and performance in *B. pumilum*. We hypothesized that (1) the two ecomorphs do not represent separate evolutionary lineages, (2) large heads (including casques and gular scales) are honest signals related to increased performance of individuals, and (3) performance is reduced when individuals have smaller characters related to signaling. To examine these hypotheses, we used mitochondrial DNA sequences to determine evolutionary history and origins of the two ecomorphs. We then compared bite force with head morphology of chameleons from open and closed habitats.

Materials and methods

Study system

The genus *Bradypodion* contains some 15 described species and another six awaiting description (Tolley et al., 2004, 2006; Tolley and Burger, 2007). All species are small (40–90 mm snout-vent length) and viviparous with allopatric distributions in South Africa, although one species ranges into adjoining Mozambique (Tolley and Burger, 2007). The genus has been the subject of much recent work on behavior (Stuart-Fox and Whiting, 2005; Stuart-Fox et al., 2006, 2007), phylogenetics (Tolley et al., 2004) and biogeography (Tolley et al., 2006, 2008). The genus *Bradypodion* appears to have originated in forested vegetation with subsequent radiations into open habitats (Tolley et al., 2008). *B. pumilum* has a limited distribution in the Cape region of South Africa in two vegetation types: "fynbos" (shrubby Mediterranean-type vegetation) and mixed (forest patches, thicket, and exotic vegetation), hereafter referred to as open and closed habitats, respectively. *B. pumilum* has only been found in these two distinctly different habitats.

Previous studies have shown that each habitat contains a distinct ecomorph which is exclusive to this habitat type (Tolley et al., 2006; Stuart-Fox et al., 2007; Tolley and Burger, 2007), a condition also known in

Author's personal copy

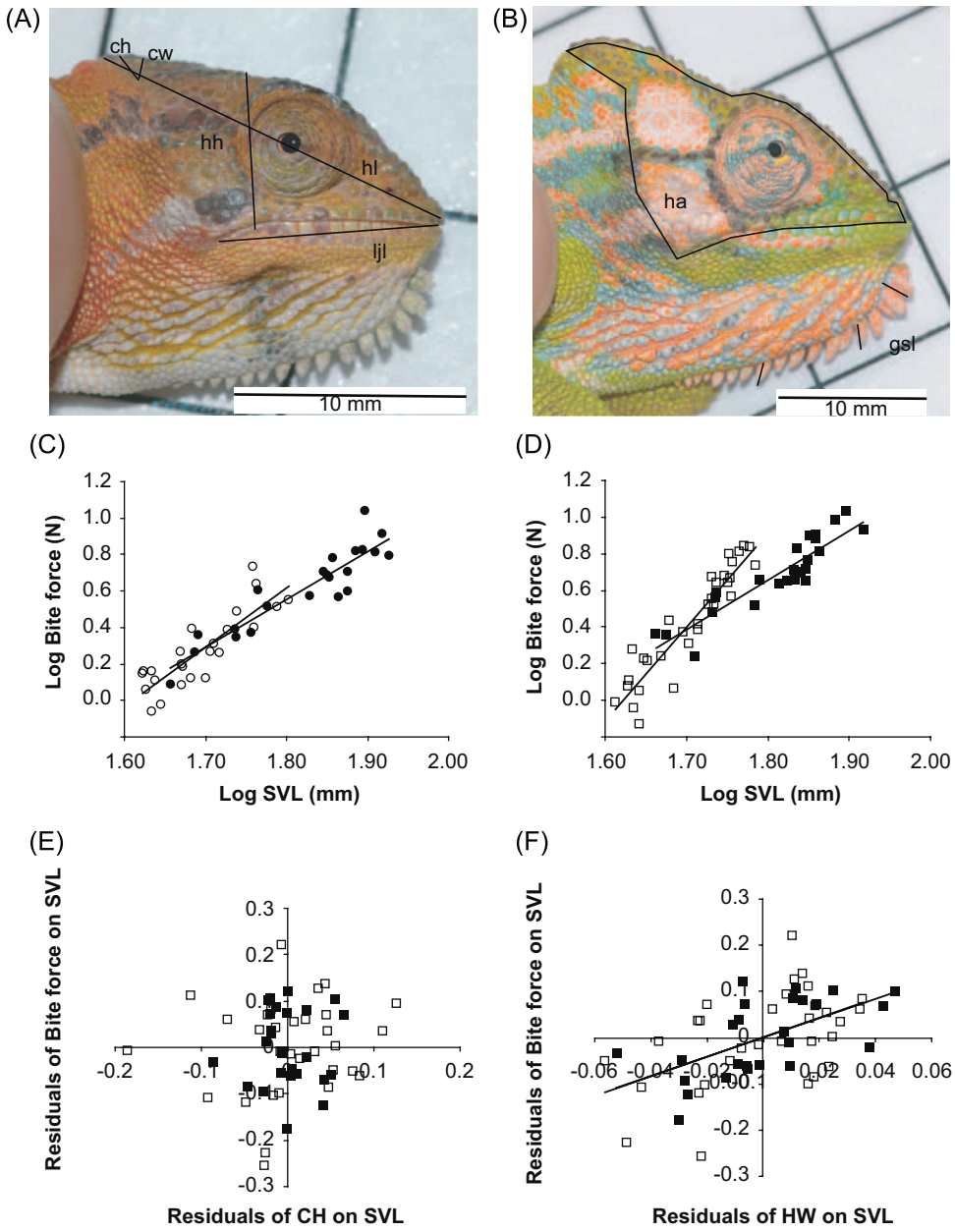


Fig. 1. *Bradypodion pumilum* males from open (A) and closed (B) habitats showing morphological measurements taken on individuals. ch casque height, cw casque width, hh head height, hl head length, ljl lower jaw length, ha head area, gsl gular scale length. (C) Increasing head size results in a harder bite for females from both habitats. (D) Males from open habitats (open squares) bite proportionately harder than males from closed habitats (closed squares). (E) After effect of body size is removed, no significant relationship remains between casque height (CH) and bite force, while (F) a strong relationship is present for head width (HW) (regression lines overlie each other). Equations for significant regression lines are given with the standard error (SE) of the slope and adjusted r^2 value.

other species of this genus (Tolley et al., 2006; Stuart-Fox et al., 2007; Tolley and Burger, 2007). For example, a preliminary reciprocal and common garden study on *B. thamnobates* and *B. melanocephalum* (although named different species, these are essentially ecomorphs of the same mitochondrial lineage; Tolley et al., 2004) has shown that these ecomorphs are not plastic, rather that their distinct morphologies are heritable (Miller and Alexander, personal communication). A detailed examination of morphologies of the ecomorphs of *B. pumilum* is currently underway (Hopkins and Tolley, unpublished data), but does not contradict these findings.

When confronted with a conspecific, male *B. pumilum* produce a display of lateral compression, raised casque, brighter coloration, extension of the gular pouch, sometimes combined with headshakes (Stuart-Fox

Author's personal copy

et al., 2007; personal observation). Displays sometimes escalate to open mouth threats and chasing followed by biting (Stuart-Fox, 2006; Stuart-Fox et al., 2006, 2007; Tolley and Burger, 2007; personal observation). Females generally respond to males with immediate aggression displays, including chasing and biting (Stuart-Fox and Whiting, 2005). Circumstantial evidence of such contests can often be found in wild caught *B. pumilum* as scars on the heads and torsos of individuals (personal observation). However, it has not been shown that force exerted during bites determines the outcome of a fight, nor that variation in bite performance is related to relative fitness, as has been shown for other species (e.g. Lappin and Husak, 2005). In examining several members of the genus, Stuart-Fox et al., (2007) showed a shift in display colors between heaths and grasslands (open) and forest (closed) habitats. Animals in closed habitats were found to have a higher UV component in their display colors, and this was interpreted as selection for signal detectability.

Field work

We conducted surveys for chameleons in open and closed habitats in the Western Cape, South Africa. When sampling chameleons, we did not discriminate for a particular size class so that adults represent a cross section of the entire range of sizes in the population. Juveniles (snout-vent length <45 mm; $n = 9$) were measured but excluded from the dataset as sex could not be determined and they are presumed not to be involved in sexual selection. Adult males were identified by eversion of hemipenes. Females were identified as individuals >45 mm with no obvious signs of hemipenes.

Genetic analyses

Species level phylogenies for the genus *Bradypodion* (Tolley et al., 2004, 2006) show that both ecomorphs of *B. pumilum* form a single well-supported, monophyletic clade. Thus, we conducted population level analyses on *B. pumilum* for a mitochondrial marker (ND2) using 27 chameleons sampled in this study plus 16 previously published sequences. Samples were obtained from across the entire range of the species, from two open and three closed sampling sites (Table 1). Laboratory protocols and primer combinations follow Tolley et al., (2004).

A spatial analysis of molecular variance (SAMOVA) was carried out using a 502 bp fragment of ND2 to determine *a priori* clustering of the sampling sites into populations (Dupanloup et al., 2002). The SAMOVA takes into consideration the geographic locations of sites, and maximizes the proportion of genetic variance among K groups (Dupanloup et al., 2002). As such, this analysis is useful for statistically differentiating between historically isolated groups that presumably represent populations. The SAMOVA was run with several different possible group structures (values of $K = 2$–6) to determine the maximum value for F_{CT} (the maximized proportion of total genetic variance due to differences between groups). The presence of isolation by distance was tested by a Mantel test (with 20,000 permutations) using Mantel for Windows v1.18 (Calvalcanti, 2005). Relationships among haplotypes were examined with a median-joining network in Network 4.1 (Bandelt et al., 1999).

Morphological measurements

Morphological variables were measured for each individual using digital callipers (accuracy 0.01 mm): snout-vent length, head height, casque height, casque width, head length, head width, head height and lower jaw length (Fig. 1A). Casques are bony parietal crests which can be "raised" by moving the head in relation to the body, but their size remains fixed whatever the orientation. Casque measurements were taken from the meeting point of the actual lateral and temporal crests to the uppermost point of the parietal crest (casque height), and from the same meeting point directly to the centre point of the parietal crest (casque width). Digital images (Nikon D70 with micro-nikkor 105 mm lens) of the lateral aspect of the head of each animal on scaled paper (Fig. 1B), were loaded into ImageJ (Rasband, 1997–2006) to estimate the bony area of the head and the mean length of three gular scales (see Fig. 1B).

Performance measurements

At collection, chameleons were placed in cloth bags and sustained at temperatures equivalent to those at which they are naturally active, 25 ± 2 °C. We followed the methodology of Herrel et al., (1999) to measure *in vivo* bite force with a Kistler piezo-electric force transducer (type 9203; ± 500 N; Kistler Inc., Switzerland), mounted into a custom-built holder. Gape angle was standardized by using plates of 5 mm. Performance measurements were repeated five times for each animal and the maximum value obtained was used in analyses.

Data analyses

We used log10 transformed head measurement variables of all chameleons in a stepwise regression to determine the traits which best predict bite force (SPSS v9.0). Prior to regression and analysis of covariance (ANCOVA), we tested the data for assumptions of normality, equality of variance and slopes, where

Author's personal copy

Table 1. GenBank accession numbers and frequency of occurrence for ND2 haplotypes from 43 *Bradypodion pumilum* sampled from 14 sites (in parentheses) in eight areas (CT – Cape Town, STB – Stellenbosch, SW – Somerset West, FR – Franschhoek, MIZ – Mizpah, KO – Kogelberg, VOG – Vogelgat and Fernkloof Nature Reserves, AG – Agulhas plain) of open and closed habitats.

Haplotype	GenBank accession no. for ND2	CT (3) 34° 00′S 18° 30′E	STB* (2) 33° 56′S 18° 49′E	SW* (1) 34° 04′S 18° 52′E	FR (2) 33° 55′S 19° 10′E	MIZ* (1) 34° 13′S 18° 57′E	KO* (1) 34° 18′S 18° 56′E	VOG (2) 34° 24′S 19° 16′E	AG (2) 34° 25′S 19° 28′E	Total (14)
		Closed	Closed	Closed	Open	Open	Open	Open	Open	
4–09	AY289891	1	0	0	0	0	0	0	0	1
16–2	FJ687162	0	0	0	0	0	0	1	0	1
CT099	AY289891	5	0	0	0	0	0	0	0	5
BF12	FJ687160	4	2	0	0	0	0	0	0	6
KB9	FJ687161	0	0	0	0	1	1	0	0	2
CT097	AY289903	0	5	1	3	8	4	0	0	21
KTH115	AY756696	0	0	0	0	0	0	0	3	3
KT061	AY756688	0	0	0	1	0	0	0	0	1
KT062	AY756689	0	0	0	0	0	0	1	0	1
KT063	AY756690	0	0	0	0	0	0	2	0	2
Total	Genetics	10	7	1	4	9	5	4	3	43
Total	Performance	0	46	2	0	24	33	0	0	105

Starred sites were used in collections of animals for performance and morphology. Coordinates for each area are given (averaged from collection sites). Totals are given for genetic and performance/morphological studies.

appropriate (Tabachnick and Fidell, 1996). Equality of slopes was tested by constructing a general linear model (in SPSS v9.0). We then determined the presence of sexual- and eco-dimorphism in these traits with ANCOVA.

Results

General

In total, 105 chameleons from five different sampling sites were measured, representing both ecomorphs: 24 females and 33 males from open habitat, 23 females and 25 males from closed habitat (see Table 1).

Genetics

The SAMOVA indicated that *B. pumilum* can best be partitioned into four populations ($F_{CT} = 0.70$, $p < 0.001$), which correspond with geography but not ecomorph (Fig. 2). When the data were separated *a priori* by sampling site, significant differences were found between Cape Town and Stellenbosch individuals despite being of the same ecomorph ($F_{ST} = 0.36$, $p < 0.01$). In addition, no significant pair-wise differences were found between Stellenbosch, Somerset West, Franschhoek, Mizpah and Kogelberg despite these sites having different ecomorphs (F_{ST} ranging from 0.00 to 0.06, p = not significant for all comparisons), showing that ecomorphs do not represent distinct populations for mtDNA. Rather than a population structure based on ecomorphs, a pattern of isolation by distance prevailed ($r = 0.71$, $p < 0.01$) suggesting that gene flow is higher between geographically close sampling sites regardless of ecomorphs present. The median-joining network shows 10 haplotypes, with no obvious pattern of ecomorph differentiation (Fig. 2). The most common haplotype is shared among ecomorphs, while closely related haplotypes are not associated with any particular ecomorph (Table 1).

Ornaments and performance

Overall, body size had a positive relationship with bite force for both sexes and ecomorphs (Tables 2 and 3; Fig. 1C and D); i.e. larger individuals have a harder bite. However, head ornaments (casque height and width and gular scale length) did not predict bite force as well as other head characters (Tables 2 and 3). The stepwise regression for morphometric head variables revealed head width (a non-ornamental character) to be the best predictor of bite force on the complete data set.

Females of both ecomorphs had significantly smaller head height, head width and bite force than males of the same ecomorph (ANCOVA statistics not shown). Females of both ecomorphs were not significantly different from each other after adjusting for size (except in gular scale length, Table 2). For males, ornaments

Author's personal copy

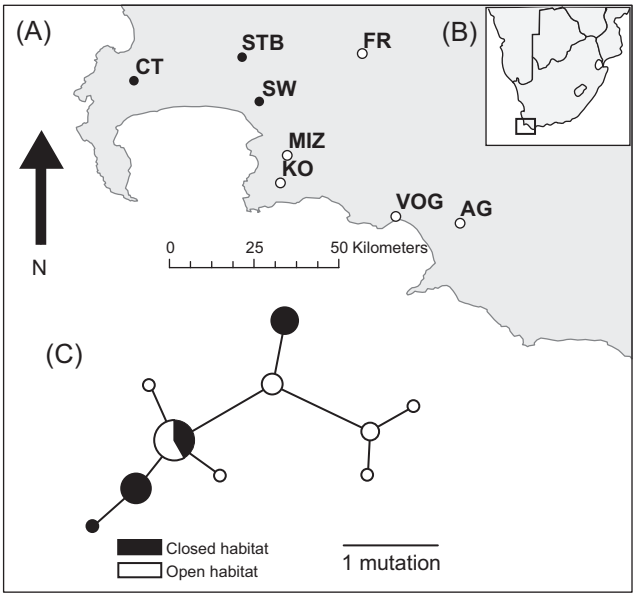


Fig. 2. (A) Sampling sites for *Bradypodion pumilum* in open (white circles) and closed habitat (black circles). CT – Cape Town, STB – Stellenbosch, SW – Somerset West, FR – Franschhoek, MIZ – Mizpah, KO – Kogelberg, VOG – Vogelgat and Fernkloof Nature Reserves, AG – Agulhas plain. (B) All sites are located in the Cape region of South Africa. (C) A median-joining network of haplotypes (ND2 mitochondrial gene) for *B. pumilum*. Haplotypes are represented by circles and show the proportion of chameleons from open habitat (white) and closed habitat (black). The area of the circle is proportional to the number of individuals with that haplotype, and the length of the connecting lines is proportional to the number of base changes between haplotypes.

(casque height and gular scale length) were larger in closed habitat ecomorphs, while non-ornaments were larger in open habitat ecomorphs (Table 2). The slopes of regression lines for males of different ecomorphs were significantly different, such that open habitat males bit proportionately harder for their size (Table 2; Fig. 1D).

In order to investigate whether head ornaments are honest signals, we calculated regressions of gular scale length, casque height and head width on bite force. The analysis was conducted for each ecomorph and sex separately. While results were significant ($p<0.001$), showing the expected allometric scaling, only head width gave a reasonable indication of bite force for both ecomorphs and casque height in closed habitat ecomorphs (Table 3). Thus, casque height of closed habitat ecomorphs is reasonably honest with respect to bite force, but in open habitat ecomorphs it is not. Removing the effects of absolute body size, by taking residuals from the regression on snout-vent length, we found no relationship between the residuals of bite force and either casque height or gular scale length (Fig. 1E; Table 3), suggesting that these characters are indeed ornaments; i.e. they do not contribute to bite force directly. When head width (the best predictor of bite force) was considered, significant positive relationships were found (Fig. 1F; Table 3), consistent with a hypothesis that variation in this non-ornamental character directly contributes to increased individual performance for both ecomorphs.

Discussion

Our molecular results reject the hypothesis of a single transition from closed to open habitats (Fig. 2, Table 2), suggesting the traits important in contributing to bite force are not constrained to a single lineage, but are environmentally linked; i.e. that they are ecomorphs and not unique evolutionary lineages. This result is consistent with previous phylogenies of the genus which suggest evolutionary radiations into open habitat (Tolley et al., 2008). Considerable phenotypic plasticity shown by some vertebrates (e.g. cichlid fish; Meyer, 1987) may allow such transitions. However, common garden experiments on dwarf chameleons suggest that this is not the case (Miller and Alexander, personal communication). Alternatively, strong selection for environmentally determined ecomorphs is known to occur within a few generations in other lizards (e.g. Losos et al., 1997; Herrel et al., 2008). In the Cape dwarf chameleon, differences in habitat are commensurate with a change in morphology and communication (Stuart-Fox et al., 2007; Tolley and Burger, 2007), a pattern consistent with the three stages common to vertebrate evolutionary radiation (Streelman and Danley, 2003).

Stuart-Fox and Moussalli (2008) hypothesized that exposure to predators may be reduced in closed habitats, reducing the need for crypsis in signaling. It follows that open habitat ecomorphs, being more cryptic and having less effective communication, require greater fighting performance. This concurs with our finding, that the open habitat ecomorph of *B. pumilum* bites proportionately harder than the closed habitat ecomorph. Tolley et al., (2008) show that species radiations in the genus *Bradypodion* are linked to shifts from closed to open habitats. This suggests that more recent lineages (or ecomorphs) in open habitats have different communication. The trade-off between the need for crypsis to avoid predation and communication to avoid fighting appears to move in both directions within this species, and results in strikingly different ecomorphs. They conform to general hypotheses in the evolution of signaling (Endler, 1992), and compare to that of different species of agamid lizards (Stuart-Fox and Ord, 2006). We interpret the open habitat ecomorph of *B. pumilum* as an example of greatly reduced traits for communication, probably as a result of predation pressure, and a subsequent investment in fighting

Author's personal copy

Table 2. ANCOVA (F-statistics and probability of equality of slopes) between ecomorphs of *Bradypodion pumilum* for separate sexes and corrected r^2 and *F*-statistics for regressions of bite force against head measurements (except bite force which was regressed against snout-vent length).

	Males				Females			
	ANCOVA *p*	*F*; *p*	r^2	*F* df = 1,54	ANCOVA *p*	*F*; *p*	r^2	*F* df = 1,43
Ornaments								
Gular scale length	0.002[a]c	1.368; 0.247	0.548	65.510	0.000[a]c	0.025; 0.876	0.674	89.001
Casque height	0.000[a]c	1.175; 0.283	0.481	50.052	0.232	3.450; 0.091	0.621	72.013
Casque width	0.008[a]c	0.030; 0.863	0.370	31.701	0.285	0.895; 0.350	0.535	50.533
Non-ornaments								
Head area	0.003[a]o	0.485; 0.489	0.812	233.872	0.962	0.094; 0.760	0.898	380.435
Head width	0.000[a]o	1.565; 0.216	0.867	352.553	0.141	2.541; 0.118	0.902	394.876
Head height	0.000[a]o	1.180; 0.282	0.837	278.202	0.643	0.172; 0.681	0.906	404.388
Head length	0.755	2.494; 0.120	0.815	238.523	0.011	0.015; 0.902	0.893	358.558
Lower jaw length	0.311	0.002; 0.968	0.821	247.546	0.117	0.514; 0.477	0.899	390.735
Bite force	[b]	30.946; 0.000	0.794	208.093	0.670	1.670; 0.203	0.868	290.497

Positive direction of difference is denoted by a 'c' or 'o' for closed and open habitats, respectively; df , degrees of freedom.
[a]Denotes ANCOVAs which are significant after Bonferroni correction.
[b]No test conducted due to inequality of slopes (see Fig. 1C).

performance. Additional insight into the evolution of ecomorph phenotype could be gained by measuring the consistency with which each transition evolves its cranial morphology in other *Bradypodion* species (see Tolley et al., 2004; Tolley and Burger, 2007).

For closed habitat ecomorphs of the Cape dwarf chameleon, our study shows that ornaments known to be effective in signaling during intraspecific competition are honest with respect to bite force performance, even though they appear not to contribute to generating bite force. Casque height, but not gular scale length, was a reasonable predictor of bite force for males and females of the closed habitat ecomorph. In general, as predicted, larger heads did produce stronger bites. However, despite this strong allometric scaling for all characters measured, *B. pumilum* showed an extensive amount of variation in ornaments when compared to non-ornamental characters. For example, the mean bite force for closed habitat ecomorph males had a range of casque heights equivalent to 58.9% of all variation in casque height. Thus, in the assessment of the fighting ability of closed habitat males, relying on ornaments alone may not accurately reflect bite force. In contrast, variation of head width, a non-ornamental character,

Table 3. Adjusted r^2 values of regressions show allometric scaling of ornamental and non-ornamental traits.

	Males				Females			
Habitat	Open df 1,29		Closed df 1,23		Open df 1,20		Closed df 1,19	
Ornaments	r^2	*F*	r^2	*F*	r^2	*F*	r^2	*F*
Gular scale	0.447	25.238	0.381	15.800	0.418	17.499	0.529	23.500
length	(0.009)	0.270	(0.006)	0.146	(0.192)	4.743	(0.000)	0.001
Casque	0.211	9.044 0.490	0.634	42.514	0.230	7.579 0.991	0.682	46.019
height	(0.017)		(0.000)	0.004	(0.012)		(0.175)	4.039
Non-ornaments								
Head width	0.859	183.460	0.876	170.068	0.737	62.680	0.901	192.022
	(0.264[a])	10.416	(0.359[a])	12.872	(0.178)	4.329	(0.386[a])	11.932

In brackets, regressions (r^2) of the same traits with bite force residuals when corrected for individual size. Relative variation in head width predicts relative bite force in chameleons, but relative variation in ornaments does not. Degrees of freedom (df) are given for each group and *F*-statistics are given following r^2 values.
[a]Denotes regressions of residuals which are significant after table-wide Bonferroni correction.

Author's personal copy

only accounted for 33.3% variation of mean bite force of the same animals. This finding is particularly interesting as previous studies (using exclusively males of closed habitat ecomorphs of *B. pumilum*) have found that casque height can be used to predict the outcome of tournaments (Stuart-Fox et al., 2006). Our results allow for the existence of cheating males, i.e. males with high casques but narrow heads and subsequently reduced bite force. It may be that males with smaller ornaments are intimidated and thus more likely to lose, and it is noteworthy that most males which initiated combat were victorious (Stuart-Fox et al., 2006).

The large intrafamilial morphological variation in chameleons (Bickel and Losos, 2002) is partly exemplified by enormous variation in casques and horns. Given the generality that larger heads bite harder, casques, horns and gular scales may all provide an advantage to the signaler by increasing the apparent surface area of the signaler's head in lateral aspect.

Several independent genera of chameleons are supported by molecular phylogenies (Townsend and Larson, 2002; Tilbury et al., 2006), yet species with different exaggerated ornaments appear independently in several of these lineages. *Chamaeleo calyptratus* Duméril and Bibron (the veiled chameleon) and *Chamaeleo jacksonii* Boulenger (Jackson's chameleon) are two extreme examples of casques and horns, respectively. Male *C. calyptratus* are known to use lateral displays of their very high casques during initiation of courtship (Kelso and Verrell, 2002), and preliminary data suggest that the height of this extreme ornament is positively correlated with bite force (Vanhooydonck et al., 2007).

B. pumilum do not have large ornaments in combination with traits that would increase bite force performance (i.e. wide heads), which is particularly apparent when the two ecomorphs are compared (Table 2). This may constitute a developmental constraint, whereby the allocation of resources to one trait produces compensatory changes in the relative sizes of other traits (Nijhout and Emlen, 1998). The spatially complex environment of a closed habitat may be thought of as inhibiting communication (Stuart-Fox and Moussalli, 2008). However, chameleons of the genus *Bradypodion* in closed habitats were found to have brighter colors with a higher UV component suggesting greater signaling efficacy (Stuart-Fox et al., 2007; Stuart-Fox and Moussalli, 2008). The balance between signaling efficacy and exposure to potential predators which have a similar visual system (i.e. birds and snakes) is thought to represent a compromise (Stuart-Fox et al., 2007). In this study, we find that individuals from closed habitats have larger ornaments, consistent with visual displays for communication. That the size of ornaments is not strictly commensurate with an increase in performance suggests that in closed habitats, chameleons signal efficiently with reduced selection for performance. Conversely, in open habitats, even brief communication by signals with a high UV component may compromise the sender to predators (Endler, 1992), suggested as an alternative hypothesis to habitat complexity by Stuart-Fox and Moussalli (2008). It follows then that selection for performance may increase while that for ornaments reduces. Support for this interpretation comes from extensive scarring found only on the open habitat ecomorphs. Although unquantified in our present study, repeating experimental tournaments, such as those conducted by Stuart-Fox (2006), with open habitat ecomorphs would greatly contribute to our understanding of the use of ornaments in signaling.

For chameleons in closed habitats, large ornaments may be used to prevent unwanted approaches (see Cuadrado, 2001; Stuart-Fox and Whiting, 2005). To date, laboratory experiments have only considered outcomes of encounters at close quarters (Stuart-Fox et al., 2006, 2007), when signaling from a greater distance may be more relevant. However, in open habitats, ecomorphs lack the ability to signal at distance but may be able to communicate in close proximity. When in close proximity, collard lizards (*Crotaphytus collaris*) make gape displays in which mouth-corner patches are revealed, and these give an accurate assessment of bite performance (Lappin et al., 2006). It is noteworthy, therefore, that chameleons also make gape displays when in close proximity (Stuart-Fox and Whiting, 2005; Stuart-Fox et al., 2006; Tolley and Burger, 2007; personal observation). *B. pumilum* has no discernable mouth-corner patch, but individuals do give a prominent display of the adductor mandibulae complex. It would be possible to determine whether this is a good predictor of bite force performance in both ecomorphs. Presumably, for chameleons in open habitat, it would be worthwhile to have signals which can be displayed at close quarters which prevent costly fights, but do not attract potential predators.

Investigations into Caribbean *Anolis* lizards have shown how different islands have produced a number of species whose morphology within a particular habitat strongly resembles those on other islands (Losos et al., 1998). For example, slow moving twig anoles live in Hispaniola and Jamaica but each is more related to the fast moving crown giant anoles on each island than they are to each other. The startling speed at which similar evolutionary outcomes evolve from single species in comparable environments has been shown through natural and laboratory experiments (Losos et al., 1997, 2000). Within the genus *Bradypodion*, *B. pumilum* is not the only species to have both open and closed habitat ecomorphs (Tolley et al., 2006; Stuart-Fox et al., 2007; Tolley and Burger, 2007). Similar morphologies from open habitats were shown to be not within the same clade, but to arise independently in many lineages

Author's personal copy

(Tolley et al., 2004, 2006, 2008). It would be revealing to discover whether each radiation into open habitat has had a commensurate change in morphology whereby bite force performance is enhanced in open habitats. Although these chameleons do not live on islands, isolation within patches of differing habitat substrates could have profound effects on these and other lizards contributing to ecologically driven adaptive radiation. Chameleons of the genus *Bradypodion* appear to be an exceptional example of the three ordered stages of vertebrate evolutionary radiation (Streelman and Danley, 2003).

Acknowledgments

We would like to thank the South African National Biodiversity Institute for providing funding for this study, and Darren Houniet, Duncan Irschick, Jenny Jackson and Michael McLeish for sampling help and logistical assistance. Anthony Herrel is thanked for constructive comments on a previous version. Thanks to Mark Johns (CapeNature, Kogelberg Nature Reserve) and Flip van Staden for access to field sites. This work was conducted under Western Cape Nature Conservation permits (001-202-00025/AAA003-00032-0035).

References

Bandelt, H.-J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48.

Bickel, R., Losos, J.B., 2002. Patterns of morphological variation and correlates of habitat use in chameleons. Biol. J. Linn. Soc. 76, 91–103.

Calvalcanti, M.J., 2005. Mantel for Windows, Version 1.18. Freeware available at 〈http://life.bio.sunysb.edu/morph/〉.

Cuadrado, M., 2001. Mate guarding and social mating system in male common chameleons (*Chamaeleo chamaeleon*). J. Zool. (London) 255, 425–435.

Dawkins, M.S., Guilford, T., 1991. The corruption of honest signalling. Anim. Behav. 41, 865–873.

Dobzhansky, T., 1951. Genetics and the Origin of Species. Columbia University Press, New York.

Dupanloup, I., Schneider, S., Excoffier, L., 2002. A simulated annealing approach to define the genetic structure of populations. Mol. Ecol. 11, 2571–2581.

Endler, J.A., 1992. Signals, signal conditions, and the direction of evolution. Am. Naturalist 139, S125–S153.

Fleagle, J.G., McGraw, W.S., 1999. Skeletal and dental morphology supports diphyletic origin of baboons and mandrills. Proc. Natl. Acad. Sci. USA 96, 1157–1161.

Herrel, A., De Grauw, E., Lemos-Espinal, J.A., 2001. Head shape and bite performance in Xenosaurid lizards. J. Exp. Zool. 290, 101–107.

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

Herrel, A., Huyghe, K., Vanhooydonck, B., Backeljau, T., Breugelmans, I., Grbac, R., Van Damme, R., Irschick, D.J., 2008. Rapid large scale evolutionary divergence in morphology and performance associated with exploitation of a different dietary resource. Proc. Natl. Acad. Sci. USA 105, 4792–4795.

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

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.

Kelso, E.C., Verrell, P.A., 2002. Do male veiled chameleons, *Chamaeleo calyptratus*, adjust their courtship displays in response to female reproductive status? Ethology 108, 495–512.

Kocher, T.D., Conroy, J.A., McKaye, K.R., Stauffer, J.R., 1993. Similar morphologies of cichlids in lakes Tanganyika and Malawi are due to convergence. Mol. Phylogenet. Evol. 2, 158–165.

Lappin, A.K., Brandt, J.F., Husak, J.F., Macedonia, J.M., Kemp, D.K., 2006. Gaping displays reveal and amplify a mechanically based index of weapon performance. Am. Naturalist 168, 100–113.

Lappin, A.K., Husak, J.F., 2005. Weapon performance, not size, determines mating success and potential reproductive output in the collared lizard (*Crotaphytus collaris*). Am. Naturalist 166, 426–436.

Losos, J.B., Warheit, K.I., Schoener, T.W., 1997. Adaptive differentiation following experimental island colonization in *Anolis* lizards. Nature 387, 70–73.

Losos, J.B., Jackman, T.R., Larson, A., de Queiroz, K., Rodríguez-Schettino, L., 1998. Historical contingency and determinism in replicated adaptive radiations of island lizards. Science 279, 2115–2118.

Losos, J.B., Creer, D.A., Glossip, D., Goellner, R., Hampton, A., Roberts, G., Haskell, N., Taylor, P., Etling, J., 2000. Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*. Evolution 54, 301–305.

Meyer, A., 1987. Phenotypic plasticity and heterochrony in *Cichlasoma managuese* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. Evolution 41, 1357–1369.

Nijhout, H.F., Emlen, D.J., 1998. Competition among body parts in the development and evolution of insect morphology. Proc. Natl. Acad. Sci. USA 95, 3685–3689.

Rasband, W.S., 1997–2006. ImageJ. US National Institutes of Health, Bethesda, Maryland, USA.

Rieppel, O., 1981. The skull and jaw adductor musculature in chamaeleons. Rev. Suisse Zool. 88, 433–445.

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

Simpson, G.G., 1953. The Major Features of Evolution. Columbia University Press, New York.

Streelman, J.T., Danley, P.D., 2003. The stages of vertebrate evolutionary radiation. Trends Ecol. Evol. 18, 126–131.

Author's personal copy

Stuart-Fox, D., 2006. Testing game theory models: fighting ability and decision rules in chameleon contests. Proc. R. Soc. London, Ser. B 273, 1555–1561.

Stuart-Fox, D., Whiting, M.J., 2005. Male dwarf chameleons assess risk of courting large, aggressive females. Biol. Lett. 1, 231–234.

Stuart-Fox, D., Ord, T.J., 2006. Sexual selection, natural selection and the evolution of dimorphic coloration and ornamentation in agamid lizards. Proc. R. Soc. London, Ser. B 271, 2249–2255.

Stuart-Fox, D., Moussalli, A., 2007. Sex-specific ecomorphological variation and the evolution of sexual dimorphism in dwarf chameleons (*Bradypodion* spp.). J. Evol. Biol. 20, 1073–1081.

Stuart-Fox, D., Moussalli, A., 2008. Selection for social signalling drives the evolution of chameleon colour change. PLoS Biol. 6, 22–29.

Stuart-Fox, D., Moussalli, A., Whiting, M.J., 2007. Natural selection on social signals: signal efficacy and the evolution of chameleon display coloration. Am. Naturalist 170, 916–930.

Stuart-Fox, D., Firth, D., Moussalli, A., Whiting, M.J., 2006. Multiple signals in chameleon contests: designing and analysing animal contests as a tournament. Anim. Behav. 71, 1263–1271.

Tabachnick, B.G., Fidell, L.S., 1996. Using Multivariate Statistics. HarperCollins College Publishers, New York.

Tilbury, C.R., Tolley, K.A., Branch, W.R., 2006. A review of the systematics of the genus *Bradypodion* (Sauria: Chamaeleonidae), with the description of two new genera. Zootaxa 1363, 23–38.

Tolley, K.A., Burger, M., 2007. Chameleons of Southern Africa. Struik, Cape Town.

Tolley, K.A., Chase, B., Forest, F., 2008. Speciation and radiations track climate transitions since the Miocene climatic optimum: a case study of southern African chameleons. J. Biogeogr. 35, 1402–1414.

Tolley, K.A., Tilbury, C.R., Branch, W.R., Matthee, C.A., 2004. Evolutionary history and phylogenetics of the southern African dwarf chameleons, *Bradypodion*. Mol. Phylogenet. Evol. 30, 354–365.

Tolley, K.A., Burger, M., Turner, A.A., Matthee, C.A., 2006. Biogeographic patterns and phylogeography of dwarf chameleons (*Bradypodion*) in an African biodiversity hotspot. Mol. Ecol. 15, 781–793.

Townsend, T.M., Larson, A., 2002. Molecular phylogenetics and mitochondrial genomic evolution in the Chamaeleonidae (Reptilia, Squamata). Mol. Phylogenet. Evol. 23, 22–26.

Vanhooydonck, B., Van Damme, R., Herrel, A., Irschick, D.J., 2007. A performance based approach to distinguish indices from handicaps in sexual selection studies. Funct. Ecol. 21, 645–652.

Wake, D.B., Larson, A., 1987. Multidimensional analysis of an evolving lineage. Science 238, 42–48.