

Rapid large-scale evolutionary divergence in morphology and performance associated with exploitation of a different dietary resource

Anthony Herrel^{*†‡}, Katleen Huyghe[†], Bieke Vanhooydonck[†], Thierry Backeljau^{†§}, Karin Breugelmans[§], Irena Grbac[¶], Raoul Van Damme[†], and Duncan J. Irschick^{||}

^{*}Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138; [†]Department of Biology, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen, Belgium; [§]Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium; [¶]Department of Zoology, Croatian Natural History Museum, Demetrova 1, HR-1000, Zagreb, Croatia; and ^{||}Department of Biology and Organismic Evolutionary Program, University of Massachusetts at Amherst, 221 Morrill Science Center, Amherst, MA 01003

Edited by Gordon H. Orians, University of Washington, Seattle, WA, and approved January 31, 2008 (received for review December 19, 2007)

Although rapid adaptive changes in morphology on ecological time scales are now well documented in natural populations, the effects of such changes on whole-organism performance capacity and the consequences on ecological dynamics at the population level are often unclear. Here we show how lizards have rapidly evolved differences in head morphology, bite strength, and digestive tract structure after experimental introduction into a novel environment. Despite the short time scale (≈ 36 years) since this introduction, these changes in morphology and performance parallel those typically documented among species and even families of lizards in both the type and extent of their specialization. Moreover, these changes have occurred side-by-side with dramatic changes in population density and social structure, providing a compelling example of how the invasion of a novel habitat can evolutionarily drive multiple aspects of the phenotype.

bite force | diet | evolution | gut structure

Recent reviews have illustrated how rapid adaptive evolution is common and may be considered the rule rather than the exception in some cases (1, 2). Experimental introductions of populations in novel environments have provided some of the strongest evidence for natural selection and adaptive divergence on ecological time scales (3–6). However, little is known about the degree to which the observed changes in morphology may affect the population structure and behavioral ecology of organisms through the mediating effects of whole-organism performance (7, 8). Consequently, our understanding of how rapid phenotypic changes affect ecological processes at the population level is limited (2, 9). Moreover, despite the fact that microevolutionary responses to environmental changes have been well documented, the unpredictability and reversibility of changes of morphological traits in fluctuating environments (10, 11) have raised questions regarding how these microscale changes can lead to the emergence of novel structures as seen on macroevolutionary scales (2).

Here we address these issues by examining the outcome of a remarkable 36-year experimental introduction with the lizard *Podarcis sicula*. In 1971 five adult pairs of this species were moved from the small islet of Pod Kopište (0.09 km²) to the nearby Pod Mrčaru (0.03 km²) by Nevo and coworkers (12). Both islets lie in the middle of the South Adriatic Sea near the larger island of Lastovo and belong to Croatia. Although the islet of Pod Mrčaru was originally inhabited by another lacertid lizard species (*Podarcis melisellenis*), repeated visits (twice yearly over the past three years, beginning in 2004) show that this species has become extinct on Pod Mrčaru. Genetic mitochondrial DNA analyses indicate that the lizards currently on Pod Mrčaru are indeed *P. sicula* and are genetically indistinguishable from lizards from the source population [supporting information (SI) Fig. 5].

Morphometric data describing head size and shape show that both males and females of the two populations differ significantly in head morphology [MANOVA; males: Wilks's $\lambda = 0.463$, $F_{9,115} = 14.81$, $P < 0.001$; females: Wilks's $\lambda = 0.425$, $F_{9,123} = 18.45$, $P < 0.001$ (Table 1 and Fig. 1)] with lizards on Pod Mrčaru having longer, wider, and taller heads than lizards on Pod Kopište (Table 1 and Fig. 1). Differences between populations are not merely the result of differences in overall size but represent distinct changes in head shape [MANCOVA with SVL as covariate; males: slopes, Wilks's $\lambda = 0.918$, $F_{6,131} = 1.96$, $P = 0.08$; intercepts, Wilks's $\lambda = 0.387$, $F_{6,132} = 34.88$, $P < 0.001$; females: slopes, Wilks's $\lambda = 0.983$, $F_{8,122} = 0.25$, $P = 0.98$; intercepts, Wilks's $\lambda = 0.754$, $F_{8,123} = 5.02$, $P < 0.001$; juveniles: slopes, Wilks's $\lambda = 0.969$, $F_{6,39} = 0.21$, $P = 0.97$; intercepts, Wilks's $\lambda = 0.498$, $F_{6,40} = 6.72$, $P < 0.001$ (Table 1 and Fig. 1)].

Differences in head size and shape also translate into significant differences in bite force between populations (males: $F_{1,44} = 4.93$, $P = 0.03$; females: $F_{1,38} = 16.94$, $P < 0.01$). Whereas the difference in bite force is the result of overall head size differences in females (ANCOVA; slopes, $F_{1,36} = 0.02$, $P = 0.91$; intercepts, $F_{1,37} = 1.55$, $P = 0.22$), in males size variation does not explain the difference in bite force (ANCOVA; slopes, $F_{1,42} = 0.25$, $P = 0.62$; intercepts, $F_{1,43} = 18.42$, $P < 0.01$).

Our data show that *P. sicula* lizards consume more plant material on Pod Mrčaru compared with the ancestral population on Pod Kopište. Analysis of stomach contents shows marked differences in diet between populations in both spring ($F_{1,204} = 22.9$, $P < 0.01$) and summer ($F_{1,74} = 103.13$, $P < 0.01$) but no differences in diet between sexes in either population ($F_{1,202} = 1.36$, $P = 0.24$). Seasonal differences in diet were significant in lizards from the introduced population (Pod Mrčaru, $F_{1,184} = 30.31$, $P < 0.01$) with plants composing between 34% (spring) and 61% (summer) of the total volume of the food eaten (Fig. 2). In contrast, plant consumption was low (7% to 4%) and did not differ seasonally for lizards from the source population (Pod Kopište, $F_{1,94} = 0.33$, $P = 0.57$). Moreover, $\approx 50\%$ of the plant matter eaten year round by lizards from Pod Mrčaru consists of items with high cellulose content such as leaves and stems (Fig. 3).

Author contributions: A.H., K.H., B.V., T.B., R.V.D., and D.J.I. designed research; A.H., K.H., B.V., I.G., R.V.D., and D.J.I. performed research; A.H., K.H., B.V., T.B., and K.B. analyzed data; and A.H., K.H., B.V., and T.B. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. EU362067–EU362088 (the 16S rDNA fragment) and EU362089–EU362110 (the 12S rDNA fragment)].

[†]To whom correspondence should be addressed. E-mail: anthony.herrel@ua.ac.be.

This article contains supporting information online at www.pnas.org/cgi/content/full/0711998105/DC1.

© 2008 by The National Academy of Sciences of the USA

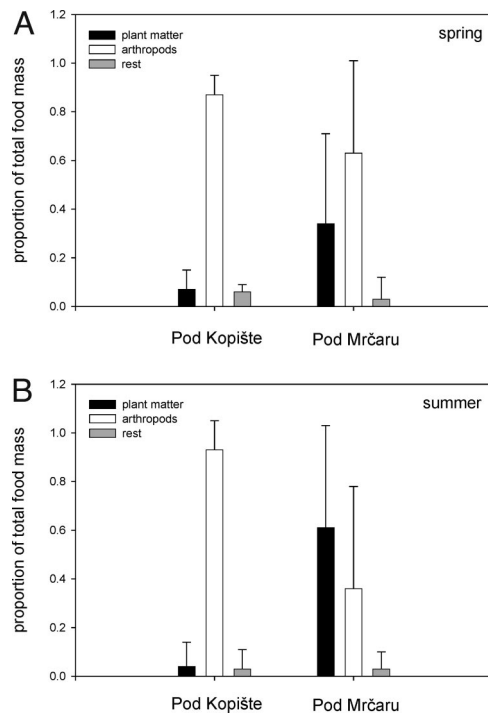


Fig. 2. Graphs illustrating differences in diet between populations in spring (A) and summer (B). Differences in the proportions of plants (black bars), invertebrate prey (white), and rest fraction (gray) are highly significant between populations. Seasonal differences in diet were highly significant on Pod Mrčaru but not on Pod Kopište. Error bars depict 1 standard deviation.

that morphological changes are specifically associated with the ability to bite hard and the increased consumption of plant matter (Table 1).

The relatively large fraction of leaves included into the diet of lizards in the introduced population of Pod Mrčaru has apparently also resulted in the evolution of cecal valves, a structure previously unreported for this species and rare in this family and scleroglossan lizards in general (13, 14, 18). Our data also add to the growing number of studies suggesting that the inclusion of plant matter into the diet of small temperate lizards may be more common than previously thought (21, 22). Moreover, our data show not only rapid, directional changes in quantitative phenotypic traits related to the inclusion of plant matter into the diet, but also the evolution of novel morphological structures on

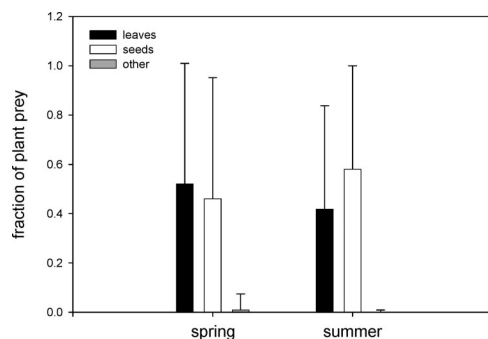


Fig. 3. Bar graph illustrating the fraction of plant prey in the diet of lizards from Pod Mrčaru consisting of leaves (black), seeds (white), and other plant material (gray). Fibrous, indigestible materials such as leaves compose a large fraction of the diet in both spring and summer. Error bars depict 1 standard deviation.

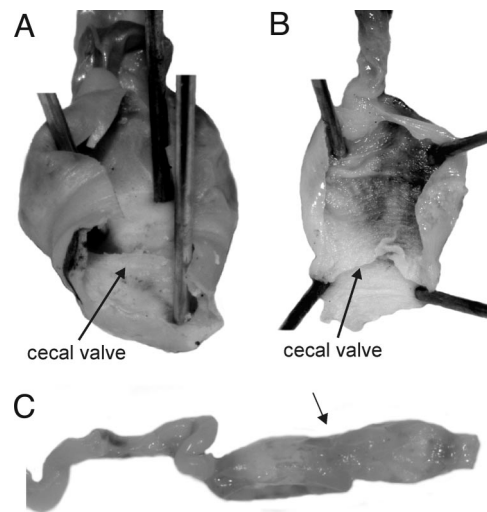


Fig. 4. Photographs illustrating the cecal valves in a male (A), a female (B), and a hatchling (C) *P. sicula* from Pod Mrčaru. Note the thick cecal wall and pronounced ridges. The arrow in C indicates the position of the cecal valve in a hatchling as seen from the outside.

extremely short time scales. Although the presence of cecal valves and large heads in hatchlings and juveniles suggests a genetic basis for these differences, further studies investigating the potential role of phenotypic plasticity and/or maternal effects in the divergence between populations are needed.

The inclusion of plant matter into diet may have had profound effects on the population structure as well. Because of the larger food base available and the increase in the predictability of the food source, lizard densities on Pod Mrčaru are much greater (0.01 versus 0.05 lizards per trap per hour, caught in unbaited traps, on Pod Kopište and Pod Mrčaru, respectively). This, in turn, likely affected the social structure, and lizards on Pod Mrčaru do no longer appear to defend territories. Moreover, changes in foraging style (browsing versus active pursuit of mobile prey) and social structure may also have resulted in the dramatic changes in limb proportions and maximal sprint speed previously documented for this population (23). Thus, our data show how rapid phenotypic changes may affect population structure and dynamics through their effect on behavioral ecology and life history of animals. They also show that rapid evolution can result in changes in both qualitative and quantitative characters.

Methods

Samples and Phylogenetic Analysis. Islands were visited in spring and summer of 2004, 2005, and 2006. Lizards were caught by noose and transported to the field laboratory or measured *in situ*. Small tail clips (± 4 mm) were taken from all individuals and stored in 100% ethanol for genetic analysis. To corroborate morphological identifications, a subset of specimens from both islands (Pod Kopište, $n = 8$; Pod Mrčaru, $n = 7$) and a set of reference specimens of *P. melisellensis* from Lastovo Island ($n = 7$) were subjected to DNA sequence analysis. Total genomic DNA was extracted by using the QIAamp DNA Mini Kit (Qiagen). Two mitochondrial DNA fragments (12S rDNA and 16S rDNA) were amplified by PCR by using the primer pairs 12SaL (5'-AAACTGGATTAGAT-ACCCCATAT-3') and 12SaH (5'-GAGGGTGACGGGCGGTGTGT-3') for the 12S fragment (modified from ref. 24) and 16Sar (5'-CCGGTCTGAACCTCAGAT-CACGT-3') and 16Sbr (5'-CGCCTGTTAACAAAAACAT-3') for the 16S fragment (25). PCRs were performed in a total volume of 25 μ l, containing 200 μ M of each dNTP, 0.2 μ M of each primer, 2.5 μ l of TaqPCR buffer ($10\times$), 1.25 units of Taq polymerase (Qiagen Taq for 12S and Sigma REDTaq for 16S), and DNA template (1 μ l for 12S and 3 μ l for 16S). The PCR protocols started with an initial DNA denaturation at 95°C (5 min) and ended with a final extension step of 5 min at 72°C. Amplification was done in 35 cycles of 95°C (1 min), 50°C (1 min), and 72°C (2 min) for 12S and 95°C (45 s), 46°C (45 s), and 72°C (90 s) for

165. PCR products were purified with the GFX PCR DNA and Gel Band Purification Kit of Amersham Biosciences and sequenced by using the Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) on an AB 3130 XL Genetic Analyzer. Sequences were edited and aligned with BioEdit software (26). The MEGA v4 (27) was used to calculate pairwise Jukes–Cantor distances and to construct a neighbor-joining tree based on a concatenated alignment of both gene fragments, including all published data of specimens for which both gene fragments were available in the GenBank database (28, 29) and with *Podarcis muralis* as outgroup (SI Fig. 5). Branch support was obtained by 1,000 bootstrap replicates.

Morphology and Performance. Snout-vent length, head dimensions, and body mass were measured for 258 adult lizards in the spring of 2004 and 2006 (Pod Kopašte, $n = 100$; Pod Mrčaru, $n = 158$). Snout-vent length and head dimensions were measured by using digital calipers, and body mass was measured by using a digital scale. Variables measured included head length, head width, head height, and lower jaw length as overall head size indicators. Additionally, biomechanically relevant measurements including the jaw outlever, the inlever for jaw opening and jaw closing, and snout length were measured as described elsewhere (30). Bite forces were measured in 100 lizards (Pod Kopašte, $n = 42$; Pod Mrčaru, $n = 58$) during the spring of 2004 using a Kistler force transducer set in a custom-built holder and connected to a Kistler charge amplifier (30, 31). Lizards were induced to bite the transducer five times, and the maximal value was retained for analysis. All morphological and perfor-

mance data were \log_{10} -transformed before analysis. Divergence rates for morphology and performance were calculated in darwins and haldanes as suggested previously (17).

Diet Analysis. A total of 330 lizards (Pod Kopašte, $n = 119$; Pod Mrčaru, $n = 211$) were stomach-flushed by using previously described methods (30), and stomach contents were stored in 70% ethanol. The stomach contents were analyzed down to order for invertebrate. Plant matter was divided in leaves, seeds, and a rest fraction. All invertebrate prey were measured (± 0.01 mm) and weighed (± 0.01 mg). Plant fractions were weighed separately, and seeds were both measured and weighed. Unidentifiable items were weighed together as a single group. The relative proportion of plant, arthropod, and the remainder were calculated per individual and arcsine-transformed before analysis.

Lizard Abundance. To get a relative assessment of lizard abundance, an array of 20 plastic cups was randomly placed across the island and left overnight. The next day lizards were retrieved from the traps, counted, measured, and released.

ACKNOWLEDGMENTS. We are indebted to Hendrik Van De Voorde (Royal Belgian Institute of Natural Sciences) for help with DNA sequencing. B.V. is a postdoctoral fellow of the Fund for Scientific Research, Flanders. This work was supported by a research program of the Fund for Scientific Research, Flanders (G.0111.06) and National Science Foundation Grant IOB 0421917 (to D.J.I.).

- Hendry AP, Kinnison MT (1999) The pace of modern life: Measuring rates of contemporary microevolution. *Evolution (Lawrence, Kans)* 53:1637–1653.
- Carroll SP, Hendry AP, Reznick DN, Fox CW (2007) Evolution on ecological time scales. *Funct Ecol* 21:387–393.
- Losos JB, Warheit KI, Schoener TW (1997) Adaptive differentiation following experimental island colonization in *Anolis* lizards. *Nature* 387:70–73.
- Phillips BL, Brown GP, Webb JK, Shine R (2006) Invasion and the evolution of speed in toads. *Nature* 439:803.
- Reznick DN, Bryga H, Endler JA (1990) Experimentally induced life-history evolution in a natural population. *Nature* 346:357–359.
- Losos JB, Schoener TW, Spiller DA (2004) Predator-induced behaviour shifts and natural selection in field-experimental lizard populations. *Nature* 432:505–508.
- Arnold SJ (1983) Morphology, performance and fitness. *Am Zool* 23:347–361.
- Huey RB, Stevenson RD (1979) Integrating thermal physiology and ecology of ectotherms—discussion of approaches. *Am Zool* 19:357–366.
- Yoshida T, Jones LE, Ellner SP, Fussman GF, Hairston NG, Jr (2003) Rapid evolution drives ecological dynamics in a predator-prey system. *Nature* 424:303–306.
- Grant PR, Grant BR (2002) Unpredictable evolution in a 30-year study of Darwin's finches. *Science* 296:707–711.
- Grant PR, Grant BR (2006) Evolution of character displacement in Darwin's finches. *Science* 313:224–226.
- Nevo E, et al. (1972) Competitive exclusion between insular *Lacerta* species (Sauria, Lacertidae). *Oecologia* 10:183–190.
- Herrel A, Vanhooydonck B, Van Damme R (2004) Omnivory in lacertid lizards: Adaptive evolution or constraint? *J Evol Biol* 17:974–984.
- Iverson JB (1980) Colic modifications in iguana lizards. *J Morphol* 163:79–93.
- McBee RH (1971) Significance of the intestinal microflora in herbivory. *Annu Rev Ecol Syst* 1:65–176.
- Troyer K (1984) Diet selection and digestion in *Iguana iguana*: The importance of age and nutrient requirements. *Oecologia* 61:201–207.
- Kinnison MT, Hendry AP (2001) The pace of modern life II: From rates of contemporary microevolution to pattern and process. *Genetica* 112–113:145–164.
- Herrel A (2007) in *Foraging Behavior in Lizards*, eds Reilly SM, McBrayer LD, Miles DB (Cambridge Univ Press, Cambridge, UK), pp 209–236.
- Bjorndal KA, Bolten AB (1992) Body size and digestive efficiency in a herbivorous freshwater turtle: Advantages of a small bite size. *Physiol Zool* 65:1028–1039.
- Bjorndal KA, Bolten AB, Moore JE (1990) Digestive fermentation in herbivores: Effect of food particle size. *Physiol Zool* 63:710–721.
- Espinoza RE, Wiens JJ, Tracy CR (2004) Recurrent evolution of herbivory in small cold-climate lizards: Breaking the ecophysiological rules of reptilian herbivory. *Proc Natl Acad Sci USA* 101:16819–16824.
- Cooper WE, Jr, Vitt LJ (2002) Distribution, extent, and evolution of plant consumption by lizards. *J Zool London* 257:487–517.
- Vervust B, Grbac I, Van Damme R (2007) Differences in morphology, performance and behaviour between recently diverged populations of *Podarcis sicula* mirror differences in predation pressure. *Oikos* 116:1343–1352.
- Kocher TD, et al. (1989) Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc Natl Acad Sci USA* 86:6196–6200.
- Simon C, et al. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann Entomol Soc Am* 87:651–701.
- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98.
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetic Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599.
- Podnar M, Mayer W, Tvrtkovic N (2005) Phylogeography of the Italian wall lizard, *Podarcis sicula*, as revealed by mitochondrial DNA sequences. *Mol Ecol* 14:575–588.
- Podnar M, Mayer W, Tvrtkovic N (2004) Mitochondrial phylogeography of the Dalmatian wall lizard, *Podarcis melisellensis* (Lacertidae). *Org Div Evol* 4:307–317.
- Herrel A, Joachim R, Vanhooydonck B, Irschick DJ (2006) Ecological consequences of ontogenetic changes in head shape and bite performance in the Jamaican lizard *Anolis lineatopus*. *Biol J Linn Soc* 89:443–454.
- 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.