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1 **Form function relationships underlie rapid dietary changes in a lizard.**

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15

16 **Abstract**

17 Macroevolutionary changes such as variation in habitat use or diet are often associated with
18 convergent, adaptive changes in morphology. However, it is still unclear how small-scale
19 morphological variation at the population level can drive shifts in ecology as observed at a
20 macroevolutionary scale. Here, we address this question by investigating how variation in cranial
21 form and feeding mechanics relate to rapid changes in diet in an insular lizard (*Podarcis siculus*) after
22 experimental introduction into a new environment. We first quantified differences in the skull shape
23 and jaw muscle architecture between the source and introduced population using 3D geometric
24 morphometrics and dissections. Next, we tested the impact of the observed variation in morphology
25 on the mechanical performance of the masticatory system using computer-based biomechanical
26 simulation techniques. Our results show that small differences in shape, combined with variation in
27 muscle architecture, can result in significant differences in performance allowing access to novel
28 trophic resources. The confrontation of these data with the already described macroevolutionary
29 relationships between cranial form and function in these insular lizards provides insights into how
30 selection can, over relatively short time scales, drive major changes in ecology through its impact on
31 mechanical performance.

32 **Keywords:** Adaptation, diet, bite force, lizard, finite element models, multibody dynamics

33 Introduction

34 Macroevolutionary changes such as changes in habitat use or diet are often associated with
35 convergent, adaptive changes in morphology. Previous studies have suggested that for
36 macroevolutionary changes to occur, directional selection driven by consistent changes in the
37 environment is needed. Consequently, [Carroll and colleagues \(2007\)](#) suggested that “macroevolution
38 may thus be nothing more than an aggregate of many small events”. Despite the initial assertion that
39 macroevolutionary patterns cannot be predicted from processes at the population level ([Eldredge
40 and Cracraft, 1980](#)), subsequent authors have demonstrated that patterns of variation among taxa
41 can be predicted using population genetics theory ([Arnold et al., 2001](#)). Moreover, in some cases,
42 variation in morphology can be rapid and may drive the subsequent evolutionary trajectory of a
43 population ([Herrel et al., 2008](#)). Indeed, variation in fitness-relevant traits has the potential to rapidly
44 drive a population across a valley of low fitness to a new adaptive peak ([Arnold, 1983; Schluter, 2000;
45 Arnold et al., 2001; Svensson and Calsbeek, 2012](#)), thus potentially driving rapid and seemingly
46 punctuated changes in morphology ([Gingerich, 1983; Eldredge, 1985](#)). Yet, for small-scale
47 population-level variation to facilitate or drive rapid shifts in ecology and potentially allow organisms
48 to reach these new adaptive peaks, this variation in morphology needs to have a significant impact
49 on function. As the link between morphology and function is often non-linear ([Koehl, 1996](#)), even
50 small differences in form may potentially give rise to significant differences in function. For example,
51 since the force output of a musculoskeletal system scales to the second power of linear dimensions,
52 small changes in head dimensions can generate significant differences in bite force ([Herrel and
53 O’Reilly, 2006](#)), which might in turn allow species to access novel resources ([Aguirre et al., 2003;
54 Herrel et al., 2004, 2006, 2008](#)).

55 Herbivory is an attractive ecological strategy that, at least in mammals, has resulted in fast species
56 diversification ([Price et al., 2012](#)) with over 38% of all mammals being considered herbivores
57 ([Atwood et al., 2020](#)). In contrast to mammals, squamate herbivores are rare with merely 2% of all
58 species being considered herbivores ([Cooper and Vitt 2002](#)). Among lizards this value may be as high
59 as 5% ([Meiri, 2018](#)), but herbivory remains an uncommon dietary niche. Among the reasons that
60 have been suggested to have prevented lizards from becoming herbivorous are their small body size
61 ([Szarski, 1962; Ostrom, 1963; Pough, 1973](#)), unspecialized dentition ([Hotton, 1955](#)), and lack of
62 complex food processing ([King, 1996](#)). However, it has since been demonstrated that even small
63 lizards can be herbivorous ([Espinoza et al., 2004](#)) and that complex cusped teeth have evolved
64 associated with an herbivorous diet ([Lafuma et al., 2021](#)). Moreover, herbivorous lizards typically
65 have higher bite forces than insectivorous species ([Herrel et al., 2004; Herrel, 2007](#)) which may allow
66 them to crop leaves from a larger plant. The herbivorous niche in lizards thus appears to be an
67 adaptive peak that is rather difficult to attain and needs to be accompanied by a suite of distinct
68 anatomical and functional specializations.

69 A notable exception to the general idea that herbivory is difficult to achieve in lizards is the
70 previously documented rapid evolution of a largely herbivorous diet in a population of Italian wall
71 lizards (*P. sicula*) roughly three decades after its introduction onto a small islet in the Adriatic ([Nevo
72 et al. 1972; Herrel et al., 2008](#)). Indeed, lizards that were introduced onto the islet of Pod Mrčaru
73 from the neighboring islet of Pod Kopište switched to a diet composed of up to 60% of plants in
74 summer and show physiological adaptations, as well as differences in their microbiome, allowing
75 them to more efficiently extract energy from a plant-based diet ([Herrel et al., 2008; Wehrle et al.,](#)

76 2020; Lemieux-Labonté et al., 2022). Moreover, in the 36 years since the introduction of these lizards
77 on Pod Mrčaru, they developed larger heads, muscles, and bite forces and changed the shape of their
78 cranium and mandible (Herrel et al., 2008; Taverne et al., 2021). As the two islets are similar in size
79 and show similar vegetation types and high lizard densities (Herrel et al., 2008) the ecological drivers
80 of the observed changes in diet remain unclear. Moreover, despite the documented changes in head
81 shape and muscle architecture it remains unclear how these lizards were able to gain a great enough
82 functional advantage to allow them to occupy a new adaptive peak in the fitness landscape (i.e., a
83 plant-based diet). Here, we use dissections, geometric morphometrics, and mechanical engineering
84 tools, including multibody dynamics analysis (MDA) and finite element modeling (FEM), to better
85 understand the functional advantages provided by the rather subtle changes in skull shape and
86 muscle architecture previously documented in these two populations (Taverne et al., 2021). We
87 demonstrate how an intricate co-evolution of skull and mandible shape with muscle architecture can
88 allow for a more efficient mechanical transfer of forces from the muscles to the jaws, and a more
89 resistant skull configuration when biting. These results provide insights into how subtle phenotypic
90 variation may give rise to fitness-relevant changes in function allowing the rapid transition towards
91 new adaptive peaks and the occupation of novel trophic niches in lizards.

92 **Material and methods**

93 *Quantification and comparison of head shape and muscular anatomy*

94 Thirteen male specimens from Pod Kopište and 14 male specimens from Pod Mrčaru were captured
95 by hand or by noose at the end of the summer of 2013. Their body size (SVL: snout-vent length) and
96 head size was measured with a Mitotoyo digital caliper (± 0.01 mm) and their bite force was
97 measured using a custom-designed bite force set-up (Herrel et al., 1999b). They were sacrificed by an
98 intramuscular injection of pentobarbital under a permit of the Croatian Ministry of the Environment.
99 Specimens were preserved in a 10% aqueous formaldehyde solution for 48h, rinsed and stored in a
100 70% aqueous ethanol solution.

101 The heads of these 27 specimens were scanned at the University of Poitiers on an Easytom micro-CT
102 (at a voxel size of 24.90 μm with the following parameters: X-ray voltage, 90 kV; X-ray intensity, 70
103 μA ; exposure time, 2000 ms; number of projections, 2500). Scans were imported into Avizo 9.0
104 (Thermo Fisher Scientific) to segment the mandible and skull which were exported as PLY files. A set
105 of landmarks and semi-landmarks on curves were used to quantify the skull and mandible shape (see
106 Taverne et al., 2021; Figure S1, Table S1). Semi-landmarks on curves were slid while minimizing the
107 bending energy and all landmarks were aligned by a Procrustes superimposition with the function
108 “gpagen” (“geomorph” package). The function “prcomp” from the *stats* package was used to run a
109 Principal Component Analysis (PCA) on the Procrustes coordinates. A MANOVA on the principal
110 components (PC) cumulatively explaining at least 85% of the variance was run to test for shape
111 differences between populations. The theoretical shapes corresponding to the extremes of the PCs
112 that distinguish populations were visualized using the functions “tps3d” and “shade3d” (“Morpho”
113 package).

114 Five additional male specimens per population were included to quantify variation in jaw muscle
115 architecture (resulting in a total 18 specimens from Pod Kopište and 19 specimens from Pod Mrčaru).
116 Each muscle bundle was extracted by dissecting the left side of the head. Muscle bundles were
117 blotted dry and weighed with a digital balance (Mettler AE100; ± 0.1 mg). The connective tissue

118 surrounding the muscles was digested by submerging the muscles in a 30% aqueous nitric acid
119 solution for 24h. Next, the nitric acid was removed, and a 50% glycerol solution was added to arrest
120 the muscle digestion. Muscle fibers were drawn under a binocular scope (Leica) with *camera lucida*
121 (see [Taverne et al. 2021](#)) and measured using Image J (Rasband 1997). Muscle volume was calculated
122 as the ratio between muscle mass and muscle density (1.06 g.cm^{-3} ; see [Mendez & Keys 1960](#)). The
123 physiological cross-sectional area (PCSA) of each muscle bundle was subsequently calculated by
124 dividing muscle volume by the mean fiber length and was subsequently corrected for pennation
125 angle. The muscles were grouped into four functional groups: the external adductors, the
126 pseudotemporalis group, the adductor posterior, and the pterygoid group ([Table S2](#)). The jaw
127 depressors and the constrictor dorsalis muscles were not considered since they are not involved in
128 jaw closing.

129 Differences in the muscular architecture (muscle volume, PCSA, fiber length) between populations
130 were tested by means of a multivariate analysis of covariance (MANCOVA) with the island as factor
131 and SVL as co-variable, using the function “mancova” from the package “jmv”. Subsequent univariate
132 ANOVAs were used to test which muscle groups were responsible for the observed differences.

133 *Multibody dynamics model design*

134 The skull and the mandible of two males of similar size, one from the island of Pod Kopište (PK), one
135 from Pod Mrčaru (PM), were segmented using Avizo 9.0 (Thermo Fisher Scientific). The 3D models of
136 the cranium and jaw of each specimen were used to build two MDA models. The location of the
137 origin and insertion of each jaw muscle was assessed based on dissections and each muscle was
138 virtually divided into several distinct muscle bundles according to the size of the muscle. The number
139 of bundles used was based on the muscle volume and area of insertion ([Table 1](#)). The coordinates of
140 the sites of origin and insertion of each muscle bundle were determined using Avizo 9.0 (Thermo
141 Fisher Scientific). The mandible was converted into a parasolid format allowing to compute the
142 inertial properties based on a bone density of 1.5 g.cm^{-3} ([Sellers & Crompton, 2004](#)). The skull
143 surface, the mandible parasolid, and the muscle bundle coordinates were imported into MSC ADAMS
144 multibody dynamic simulation software (Santa Ana, CA, USA). The muscle bundles were modelled as
145 contractile springs. Where necessary, muscle bundles were wrapped around the bone to increase the
146 accuracy of modelling ([Gröning et al., 2013](#); [Fig. 1](#)). Muscle PCSAs were corrected by both the
147 pennation angle and the typical 10% loss in muscle volume due to tissue preservation ([Kikuchi &](#)
148 [Kuraoka, 2014](#)). The maximum force of each muscle ([Table 1](#)) was calculated by multiplying the PCSA
149 by an intrinsic muscle stress of 40 N/cm^2 ([Gröning et al., 2013](#)). The MDA models simulated opening
150 and closing of the jaw assuming maximal activation of the bundles when the system was at
151 equilibrium. A food particle was created and aligned perpendicularly to the toothrow of the upper
152 jaw to generate a reaction force due to jaw closing (simulated bite force). To ensure consistency in
153 the output bite force generated by the MDA models, the location of the contact between the food
154 particle and the teeth was prescribed to match the location of the contact between the teeth and the
155 plates of the force transducer used to measure *in vivo* bite force in the field ([Taverne et al., 2020](#)). To
156 run further simulations, the location of the item was then standardized at the middle of the maxillary
157 toothrow, as observations revealed that lizards typically crush prey at that location ([Taverne et al.,](#)
158 [2022](#)). The stiffness of the food particle was intentionally set beyond the hardness of prey typically
159 consumed by the lizards to ensure that the gape angle did not change during a biting simulation and
160 to obtain the maximal bite force.

161 *Finite element model design*

162 Two finite-element meshes consisting of about 1.5 million tetrahedral elements (Pk: 1,527,268
163 elements; PM: 1,321,278 elements) were generated in Avizo and imported into ANSYS (ANSYS, Inc.,
164 Canonsburg, PA, USA) for finite element analysis. Bone was assumed to have isotropic homogenous
165 material properties with a Young's modulus of 17 GPa and a Poisson's ratio of 0.3 (Cowin, 2001;
166 Rayfield, 2005). The meshes were constrained at the ventral base of the quadrates (the right side in
167 all three directions, the left in A-P and D-V only) and at two symmetrical bite points in the D-V
168 direction (Fig. 1). The coordinates of these locations were directly exported from ADAMS, with the
169 three-dimensionally resolved forces exported from the MDA solutions then directly applied onto the
170 skull model. The quadrato-jugal and the epipterygoid-parietal ligaments were modelled as tension-
171 only links with a stiffness of 250 N/mm² and a cross-sectional area of 1mm² (Fig. S2). Sensitivity tests
172 were carried out using the Pod Kopište model biting at a 20° gape to quantify the impact of the
173 presence / absence and variation in stiffness of the ligaments (50, 250, and 500 N/mm²) on the stress
174 distribution (Figs. S3, S4). The stress values associated with each element of the mesh were exported
175 into an element table for postprocessing.

176 *Simulations*

177 Four different MDA and FEA models were built: two "natural" models in which the specimens were
178 modelled with their own musculature (PK_PK and PM_PM); and two "theoretical" models in which
179 muscle PCSAs were swapped (i.e., PK_PM: the morphology of Pod Kopište with the musculature of
180 Pod Mrčaru, and PM_PK the morphology of Pod Mrčaru with the musculature of Pod Kopište). These
181 models allowed us to test the impact of changes in cranial morphology and muscle anatomy on the
182 calculated bite force by comparing the results of the simulations. The calculated bite force in each of
183 the four models was calculated for 10 different gape angles, from 0° (closed jaw) to 45° (maximum
184 gape typically attained at the onset of fast closing, Taverne et al., 2022), thereby effectively varying
185 the size of the prey item. For each MDA model, the conversion rate of the total muscle force into bite
186 force was calculated at every gape tested by dividing bite force by the total muscle force and was
187 used to assess the efficiency of a muscle to translate intrinsic muscle force into bite force. The total
188 amount of change in bite force between the two natural models was quantified as it provides direct
189 information on how much the mechanical properties of the masticatory system of the two specimens
190 differ. The amount of change in bite force due to the change in muscle anatomy or to the change in
191 cranial morphology alone was also quantified. This allowed us to estimate the relative contribution
192 (in %) of changes in cranial morphological or in muscular anatomy to generate the observed
193 differences in bite force between the specimens. The role of each muscle in bite force generation
194 was assessed by running MDAs with only one muscle activated at a time.

195 The results of the MDA models were then imported into ANSYS, after having been multiplied by five
196 to obtain realistic absolute loads corresponding to *in vivo* bite forces (i.e. the calculated bite forces
197 were five times lower than the *in vivo* forces). For each model, FEAs were performed at three gape
198 values that mimicked three ecologically relevant scenarios: biting a thin object like a leaf (0°), biting a
199 prey of medium size (20°) and biting a large prey item or a conspecific during for example male-male
200 interactions (45°). Values of stress in each skull element were saved. Specifically, we focused on the
201 von Mises' stress (vMS) and recorded the mean stress values for the whole skull and the variation in
202 the stress along the skull. To do so we divided the geometry into 10 segments of equal length (Fig. 1)

203 and averaged the vMS values within each section. We then calculated the ‘advantage’ of the PM
204 morphology relative to the PK morphology as the percentage of increase/decrease of the mean vMS
205 accumulation along the skull. Finally, we estimated the homogeneity of the distribution of stress by
206 calculating the Shannon’s entropy (Shannon, 1948) based on the density distribution of vMS values.
207 A higher entropy reflects a low redundancy and a high disparity in the data. Thus, the ‘advantage’
208 provided by the PM morphology was estimated as the proportional difference in entropy between
209 the two models.

210 **Results**

211 *Morphological differences*

212 Lizards from the two islets differ in snout-vent length (Pod Kopište: 62.3 ± 3.7 mm for males; $58.2 \pm$
213 3.0 mm for females; Pod Mrčaru: 68.3 ± 3.2 mm for males; 62.72 ± 2.9 for females), head length (Pod
214 Kopište: 14.9 ± 0.7 mm for males; 13.0 ± 0.6 mm for females; Pod Mrčaru: 16.22 ± 0.8 mm for males;
215 13.8 ± 0.6 for females), and bite force (Pod Kopište: 17.3 ± 4.6 N for males and 9.6 ± 1.9 N for
216 females; Pod Mrčaru: 27.01 ± 3.2 N for males and 14.8 ± 2.7 N for females).

217 The MANOVA including the first nine axes of the PCA carried out on skull shape (cumulative variance:
218 85.2%) revealed an effect of island (Wilks’ lambda = 0.22; $F_{1,25} = 6.861$; $P < 0.001$), especially on PC3
219 (variance = 9.3%; $F_{1,25} = 26.48$; $P < 0.001$) and PC6 (variance = 3.3%; $F_{1,25} = 4.83$; $P = 0.037$). The
220 theoretical shape deformation along PC3 showed that the population of PM had a slightly shorter
221 snout and a more curved quadrate (Figure S5). The MANOVA including the first 9 axes of the PCA
222 carried out on mandible shape (cumulative variance: 85.0%) also revealed an effect of island (Wilks’
223 lambda = 0.40; $F_{1,25} = 2.86$; $P = 0.029$), especially along PC1 (variance = 24.4%; $F_{1,25} = 7.50$; $P = 0.011$).
224 The theoretical shape deformation along PC1 showed that the mandible of the PM population was
225 overall more ventrally curved (Figure S5). The coronoid was medially and caudally thicker, the lateral
226 crest, which serves as the insertion site of external adductors was wider, the retroarticular process
227 shorter, and the joint surface area larger.

228 The MANCOVA carried out on the muscle variables detected significant differences between islands
229 (Table 2). All variables (muscle mass, fiber length, and PCSA) were greater in specimens from Pod
230 Mrčaru. When accounting for body size, specimens from Pod Mrčaru had heavier external adductors
231 and pterygoid muscles, shorter fibers in the AMP, and a greater PCSA of the external adductors and
232 the AMP (Table 3).

233 *Mechanical basis of variation in bite force*

234 The MDAs showed that models with the musculature of the PM specimen always resulted in higher
235 bite forces, and that models with the shape of PM individuals resulted in higher bite forces
236 irrespective of variation in muscle architecture (Fig. 2, Table 4). This held irrespective of the gape
237 tested, and was even exacerbated with an increase in gape. Simulated bite force reached two peaks
238 in the four models, at closed gapes (0° or 5° in PK_PM) and wide gapes (45°) and was minimal at a
239 gape of around $30^\circ / 35^\circ$. Knowing that the total bite force of the PK and the PM individuals modeled
240 in the present study was 19.1 N and 25.0 N respectively, the simulations showed that the conversion
241 rate of the PM morphology is always higher than in the PK morphology (Fig. 2). The total amount of
242 change in simulated bite force between the two natural models varied between 30.5% (0°) and

243 37.5% (40°). On average, the relative contribution from the change in skull shape to total variation in
244 bite force increased with gape and varied between 2.41% (5°) and 12.49% (45°), the remainder being
245 explained by variation in muscle architecture (Fig. 3).

246 In all models, the external adductors contributed the most to bite force (on average approximately
247 50%), then the pseudotemporalis (35%), and the pterygoids (15%) (Fig. S6, Fig. S7, Table S3). The
248 relative contribution of the muscles varied with gape; the contribution of the external adductors
249 decreased with gape, while that of the pterygoid increased. The pseudotemporalis group had the
250 highest conversion rate of muscle force into bite force (approximately 30% on average), followed by
251 the external adductors (22%) and the pterygoids (12%) (Figure S7). The conversion rate of the
252 adductors decreased with gape in all models, while the conversion rate of the pterygoids increased,
253 and that of the pseudotemporalis decreased from gape 0° to 30° and increased from 30° to 45°.
254 Compared to the morphology of PK, the PM morphology was associated with higher conversion rates
255 of the adductors for all gapes, of the pseudotemporalis for gapes wider than 10°, and for the
256 pterygoids for gapes wider than 30°.

257 *Structural performance of the skull*

258 The comparison of the two natural models showed that, on average, the von Mises stress magnitude
259 (vMS) in the cranium of PM_PM model was higher than in that in the PK_PK model (from 6% to 17%)
260 (see Fig. 4). Although PM_PM showed lower vMS magnitudes (except for the two most posterior
261 sections of the skull) at a 0° gape, more stress was detected at 20° and 45°. Interestingly, the amount
262 of vMS observed significantly increased with gape in PM_PM, whereas this was not the case in
263 PK_PK. Specifically, the increase in vMS in the PM_PM natural model affected the whole skull (Fig. 4).
264 An increase of 2% to 6% was also observed in the overall entropy in the PM_PM model (Table 5).

265 The PM morphology enabled the mean vMS to decrease by 15% compared to a PK skull model with
266 the PM musculature. The advantage provided by the PM morphology, estimated in percentage of
267 loss in vMS, increased at low gape and was more pronounced in the anterior part of the skull than
268 the posterior part (see Fig. 5). Indeed, the advantage of a PM morphology was 13% to 41% in the
269 snout, 9% to 20% in the region of the jugal, and became null or slightly negative in the two most
270 posterior regions (where stress magnitude increased). Specifically, the unloading allowed by the PM
271 skull morphology concerned the maxilla, the vomer, the frontal, the anterior part of the pterygoid,
272 the epipterygoid, the parietal, and the quadrate. On the contrary, the areas that showed higher
273 stress magnitudes included the wings of the prootic and the posterior tip of the pterygoid. Although
274 less obvious when reaching a 45° gape, this pattern of stress distribution was similar at all gapes (Fig.
275 5). On average, the vMS magnitude increased (from 19% at 0° to 27% at 45°) when a PM musculature
276 rather than a PK musculature was applied to a given morphology. In all cases, the overall level of
277 entropy increased when a PM musculature rather than a PK musculature was applied on a given
278 morphology (from 6% at 0° to 7% at 45°). The PM morphology provided only a small advantage in
279 reducing overall entropy (differences < 5%; Table 5).

280 **Discussion**

281 A significant difference in cranial anatomy and muscle architecture exists between the individuals
282 from Pod Kopište and Pod Mrčaru. The latter showing stronger jaw adductors associated with their
283 larger size and omnivorous diet. Lizards from Pod Mrčaru are bigger than those from Pod Kopište,

284 but the variation in muscle cross-sectional area is not only due to differences in size. This is in line
285 with the results of a previous study comparing multiple populations of *Podarcis* lizards (Taverne et al.
286 2021). Our results suggest that other factors may impact the differences in muscle architecture. For
287 example, lizards from Pod Mrčaru have stronger pterygoid muscles which have a low moment arm at
288 low gape (Herrel et al. 1999 a,b). As plant consumption typically involves biting at low gape, this
289 suggests that diet might not underpin the differences in the cross-sectional area of pterygoid muscles
290 observed between lizards from the two populations. We rather suggest that biting in the context of
291 intraspecific interactions may better explain this difference. The density of lizards on Pod Mrčaru is
292 roughly five times higher than on Pod Kopište (Herrel et al., 2008; Vervust et al., 2009), increasing the
293 probability of encounters and aggressive interactions. Biting congeners involves biting at large gape
294 which may drive the observed differences in the pterygoid muscle (Donihue et al., 2016). Populations
295 also differed in cranial and mandibular shape. Our results show that lizards on Pod Mrčaru have a
296 mandible that is more ventrally curved, with a wider lateral insertion area for adductor muscles, and
297 a more robust coronoid. The results for skull morphology are less clear and mainly show differences
298 in the curvature of the quadrate and a shorter snout in animals from Pod Mrčaru. The fact that the
299 morphological differences in the cranium are less clear is likely a consequence of the multiple
300 functions that the cranium fulfills (e.g., protection of the central nervous system and of the sensory
301 organs), and the associated constraints and trade-offs. Non-adaptive processes including founder
302 effects or genetic drift after introduction may also have driven the phenotypic differences between
303 the two populations observed here (Kolbe et al. 2012).

304 Our modeling results demonstrate that differences in bite force between the two morphotypes are
305 principally driven by the difference in the total muscle PCSA (Table 2). The *in-silico* estimates of bite
306 force generated by the MDA simulations suggested that the lizards from Pod Mrčaru bite harder than
307 those of Pod Kopište. Yet, subtle differences in cranium and mandible shape also contributed to the
308 observed variation in bite force between the two populations. The comparison of the natural and
309 hybrid models showed that the morphology of Pod Mrčaru enables greater bite forces to be
310 generated in all cases. Moreover, the correlation between bite force and gape parallels the observed
311 relation between conversion rate and gape (Fig. 2). Hence, variation in bite force at different gape
312 angles depends on the proportion of muscle force converted into bite force, which in turn is
313 impacted by head shape. This is further supported by the relative contribution of musculature and
314 morphology to the differences in bite force. Consequently, the relatively subtle shape changes in the
315 mandible and cranium between these two recently diverged populations are responsible for a
316 considerable part of the variation in bite force. This is achieved by modulating the lever-arms
317 associated with the muscle bundles that depend on the variation in skull and mandible geometry as
318 well as differences in muscle size and cross-sectional area.

319 The comparison of the natural models PK_PK and PM_PM highlighted differences in structural
320 performance between the phenotypes of the two populations and revealed that the phenotype of
321 Pod Mrčaru accumulates more stress. Given that the PM model includes a greater total muscle PCSA,
322 this is expected. Moreover, our models confirm that applying greater forces on a given morphology
323 increases the level of VM stress over the skull. But the magnitude of differences in stress due to
324 musculature is greater (19% to 27%) than that observed between the natural models (from 6% to
325 17%) suggesting that morphology itself might help dissipate stress. Indeed, the PM morphology
326 conferred a clear advantage in dissipating stress compared to the PK morphology (Fig. 5). Similarly,
327 the comparison of the natural models revealed that the phenotype of the Pod Mrčaru individuals had

328 a greater level of overall entropy compared to that of Pod Kopište (from 2% at 0° to 6% at 45°).
329 Again, this is mainly due to an increase in the total muscle force with the application of a PM
330 musculature to a given morphology leading to an overall increase in entropy (from 6% at 0° to 7% at
331 45°). Interestingly, our results suggest that an increase in the total muscle force is accompanied with
332 an increased disparity in the distribution of the stress over the skull, yet the phenotype of Pod
333 Mrčaru maintained a more homogenous stress distribution.

334 Unexpectedly, the PM morphology was more advantageous than the PK morphology in producing
335 bite force at wide gapes (as revealed by the MDA simulations), whereas it is more advantageous in
336 dissipating stress at lower gapes (as revealed by the FEA simulations). Although this might first seem
337 contradictory, we propose that these two results are not incompatible. We rather think that
338 selection primarily acts upon the performance of the musculoskeletal system (i.e., bite force) within
339 the limits imposed by the intrinsic capacity of the system to dissipate the associated stress and strain.
340 Therefore, the MDA results suggest that the evolution of skull morphology towards a Pod Mrčaru
341 phenotype enabled an optimization of bite force at wide gapes. This is likely driven by intraspecific
342 competition, enhanced by a five-fold increase in population density on Pod Mrčaru (Herrel et al.
343 2008, Vervust et al. 2009). The consumption of hard and fibrous material like plant matter requires
344 repeated and frequent biting (Herrel, Verstappen & De Vree, 1999) to reduce the item into smaller
345 and more easily digestible bite-size pieces (Bjorndal et al. 1990; Bjorndal & Bolten, 1992). The
346 structural advantage provided by the PM morphology at low gapes might be important in this
347 context. Indeed, the repeated loading occurring at low gapes experienced by the skull while feeding
348 on plants is likely to be particularly constraining and might represent an important selective agent
349 driving variation in skull shape. A scenario in which head shape in insular *Podarcis* lizards is initially
350 driven by intraspecific competition, and maintained by functional demands associated with resource
351 use, would be in line with the results of Donihue and collaborators (2016) concerning intraspecific
352 variation in morphology and performance in another *Podarcis* species.

353 Previous comparative studies on the colonization of *Podarcis melisellensis* and *Podarcis siculus* in the
354 Adriatic archipelago and their morphological evolution in relation to variation in ecological contexts
355 (Taverne et al. 2020, 2021) suggested that relationships between form and function were similar at
356 different levels of integration, whether between isolated populations or between species, and even
357 when accounting for phylogenetic relationships. Specifically, convergent evolution of muscle
358 architecture and head morphology were found in similar ecological contexts. Here, we demonstrate
359 that these form-function relationships can evolve on ecological time scales and be associated with an
360 optimization of the mechanical output of the masticatory system. Consequently, subtle
361 morphological variation may accumulate over time and give rise to macroevolutionary patterns.

362 *Future directions*

363 Further efforts might help make the finite element models even more realistic and circumvent some
364 of the current limitations. First, the simulations revealed an unrealistic accumulation of vMS at the
365 very back of the skull (Figs. 4, 5) at the most lateral region of the neurocranium (paraoccipital process
366 of the exoccipital). In the present study, the joint between the lateral process of the exoccipital and
367 the quadrate was modeled as connected and fixed, hence possibly explaining why abnormal stress
368 concentrations are located in this area instead of being more homogeneously distributed. It would be
369 useful to model a ligamentous connection between these bones allowing a better stress dissipation.

370 Second, bone tissue was modeled as an isotropic material here, although it is known to be
371 anisotropic (Moazen et al. 2015). A precise quantification of the bony material properties throughout
372 the skull (Young's modulus) should be undertaken using a nano-indentation approach. A third way to
373 gain accuracy would be to model the sutures and the osteoderms, since they have been suggested to
374 impact how loads are distributed and dissipated (Moazen et al. 2008; Costantini et al. 2010; Jones et
375 al. 2017).

376 *Conclusions*

377 Our results show significant differences in skull form and function in individuals of two populations of
378 *P. siculus*. Following the introduction into a novel environment, the population on Pod Mrčaru rapidly
379 evolved a largely herbivorous diet. Our analyses of shape and function provide a mechanistic
380 underpinning of the link between relatively subtle differences in morphology and the observed
381 changes in ecology. Our results demonstrate that relationships between form and function may drive
382 variation in fitness-relevant performance traits resulting in changes in trophic ecology over a
383 relatively short time scale.

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391 **Author contributions**

392 MT, MJF and AH designed the study. MT, HD, RB, DL, ZT, ACF and AH generated the raw data. MT,
393 PW and MJF built the models. MT analyzed the data and ran the models. All authors were involved in
394 the interpretation of the results, the writing, and the revision of the manuscript.

395 **Data Accessibility**

396 doi:10.5061/dryad.866t1g1vk

397 sharing link: [https://datadryad.org/stash/share/uy5-](https://datadryad.org/stash/share/uy5-FARWE09mXDedc1SGivCLqz8aqQU6fK16_Ak9L_Y)
398 [FARWE09mXDedc1SGivCLqz8aqQU6fK16_Ak9L_Y](https://datadryad.org/stash/share/uy5-FARWE09mXDedc1SGivCLqz8aqQU6fK16_Ak9L_Y)

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400 **References**

401 Aguirre LF, Herrel A, Van Damme R, Matthysen E. 2003. The implications of food hardness for diet in
402 bats. *Functional Ecology* 17:201-212.

403 Arnold SJ. 1983. Morphology, performance, and fitness. *American Zoologist* 23: 347-361.

404 Arnold SJ, ME Pfrender, AG Jones. 2001. The adaptive landscape as a conceptual bridge between
405 micro- and macroevolution. *Genetica* 112-113: 9-32.

406 Atwood TB, SA Valentine, E Hammill, DJ McCauley, EMP Maddin, KH Beard, WD Pearse. 2020.
407 Herbivores at the highest risk of extinction among mammals, birds, and reptiles. *Science Advances* 6:
408 eabb8458.

409 Bjorndal KA, Bolten AB. 1992. Body size and digestive efficiency in an herbivorous freshwater turtle:
410 advantages of small bite size. *Physiological Zoology* 65: 1028-1039.

411 Bjorndal KA, Bolten AB, Moore JE. 1990. Digestive fermentation in herbivores: effect of food particle
412 size. *Physiological Zoology* 63: 710-721.

413 Carroll SP, AP Hendry, DN Reznick, CW Fox. 2007. Evolution on ecological timescales. *Functional*
414 *Ecology* 21: 387-393.

415 Cooper WE, Vitt LJ. 2002. Distribution, extent, and evolution of plant consumption by lizards. *Journal*
416 *of Zoology* 257: 487-517.

417 Costantini D, Alonso ML, Moazen M, Bruner E. 2010. The relationship between cephalic scales and
418 bones in lizards: a preliminary microtomographic survey on three lacertid species. *The Anatomical*
419 *Record: Advances in Integrative Anatomy and Evolutionary Biology* 293(2): 183-194

420 Cowin SC. (Ed.). 2001. Bone Mechanics Handbook (2nd ed.). CRC Press, Boca Raton, Florida.

421 Donihue CM, Brock KM, Foufopoulos J, Herrel A. 2016. Feed or fight: testing the impact of food
422 availability and intraspecific aggression on the functional ecology of an island lizard. *Functional*
423 *Ecology* 30: 566-575.

424 Eldredge N, J Cracraft. 1980. Phylogenetic Patterns and the Evolutionary Process. Method and Theory
425 in Comparative Biology. New York: Columbia University Press.

426 Eldredge N. 1985. Time Frames: The evolution of punctuated equilibria. Princeton: Princeton
427 University Press.

428 Espinoza RE, Wiens JJ, Tracy CR. 2004. Recurrent evolution of herbivory in small, cold-climate lizards:
429 breaking the ecophysiological rules of reptilian herbivory. *Proc. Natl Acad. Sci. USA* 101: 16819–
430 16824.

431 Gingerich PD. 1983. Rates of evolution: effects of time and temporal scaling. *Science* 222: 159-161.

432 Gröning F, Jones MEH, Curtis N, Herrel A, O’Higgins P, Evans SE, Fagan MJ. 2013. The importance of
433 accurate muscle modelling for biomechanical analyses: a case study with a lizard skull. *Journal of the*
434 *Royal Society Interface* 10: 20130216.

435 Herrel A. 2007. Herbivory and foraging mode in lizards. In: Lizard Ecology: The evolutionary
436 consequences of foraging mode. (S.M. Reilly, L.D. McBrayer and D.B. Miles, Eds.) Cambridge
437 University Press, Cambridge.

438 Herrel A, Aerts P, Fret J, De Vree F. 1999a. Morphology of the feeding system in Agamid lizards:
439 ecological correlates. *The Anatomical Record* 254: 496-507.

440 Herrel A, Spithoven L, Van Damme R, De Vree F. 1999b. Sexual dimorphism of head size in *Gallotia*
441 *galloti*: testing the niche divergence. *Functional Ecology* 13: 289-297.

442 Herrel A, Verstappen M, De Vree F. 1999. Modularity complexity of the feeding repertoire in scincid
443 lizards. *Journal of Comparative Physiology A* 184: 501-518.

444 Herrel A, B Vanhooydonck, R Van Damme. 2004. Omnivory in lacertid lizards: adaptive evolution or
445 constraint? *Journal of Evolutionary Biology* 17: 974-984.

446 Herrel A, Huyghe K, Vanhooydonck B, Backeljau T, Breugelmans K, Grbac I, Van Damme R, Irschick DJ.
447 2008. Rapid large-scale evolutionary divergence in morphology and performance associated with
448 exploitation of a different dietary resource. *Proceedings of the National Academy of Sciences* 105:
449 4792-4795.

450 Herrel A, JC O'Reilly. 2006. Ontogenetic scaling of bite force in lizards and turtles. *Physiol. Biochem.*
451 *Zool* 79: 31-42

452 Herrel A, R Joachim, B Vanhooydonck, D.J. Irschick. 2006. Ecological consequences of ontogenetic
453 changes in head shape and bite performance in the Jamaican lizard *Anolis lineatopus*. *Biological*
454 *Journal of the Linnean Society* 89: 443-454

455 Hotton III N. 1955. A survey of adaptive relationships of dentition to diet in the North American
456 Iguanidae. *American Midland Naturalist* 88:114.

457 Jones MEH, Gröning F, Dutel H, Sharp A, Fagan MJ, Evans SE. 2017. The biomechanical role of the
458 chondrocranium and sutures in a lizard cranium. *Journal of the Royal Society Interface* 14: 20170637.

459 Kikuchi Y, Kuraoka A. 2014. Differences in muscle dimensional parameters between non-formalin-
460 fixed (freeze-thawed) and formalin-fixed specimen in Gorilla (*Gorilla gorilla*). *Mammal Study* 39: 65-
461 72.

462 King GM. 1996. Reptiles and herbivory. Springer Science & Business Media.

463 Koehl MAR. 1996. When does morphology matter. *Annual Review of Ecology and Systematics* 27:
464 501-542.

465 Kolbe JJ, Leal M, Schoener TW, Spiller DA, Losos JB. 2012. Founder effects persist despite adaptive
466 differentiation: a field experiment with lizards. *Science* 335: 1086-1089.

467 Lafuma F, Corfe IJ, Clavel J, Di-Poï N. 2021. Multiple evolutionary origins and losses of tooth
468 complexity in squamates. *Nature communications* 12: 1-13.

469 Lemieux-Labonté V, C Vigliotti, Z Tadic, B Wehrle, P Lopez, E Bapteste, F-J Lapointe, D German, A
470 Herrel. 2022. Proximate drivers of population-level lizard gut microbial diversity: impacts of diet,
471 insularity, and local environment. *Microorganisms* 10: 1550.

472 Meiri S. 2018. Traits of lizards of the world: Variation around a successful evolutionary design. *Global*
473 *Ecology and Biogeography* 27: 1168-1172.

474 Mendez J, Keys A. 1960. Density of fat and bone mineral of mammalian muscle. *Metabolism-clinical*
475 *and Experimental* 9: 184-188.

476 Moazen M, Curtis N, O'Higgins P, Jones MEH, Evans SE, Fagan MJ. 2008. Assessment of the role of
477 sutures in a lizard skull: a computer modelling study. *Proceedings of the Royal Society B* 276: 39-46.

478 Moazen M, Peskett E, Babbs C, Pauws E, Fagan MJ. 2015. Mechanical properties of calvarial bones in
479 a mouse model for craniosynostosis. *PlosOne* 10: e0125757.

480 Nevo E, Gorman G, Soulé M, Yang SY, Clover R, Jovanović V. 1972. Competitive exclusion between
481 insular *Lacerta* species (Sauria, Lacertidae). Notes on experimental introductions. *Oecologia* 10: 183-
482 190.

483 Ostrom JH. 1963. Further comments on herbivorous lizards. *Evolution* 17: 368-369.

484 Pough FH. 1973. Lizard energetics and diet. *Ecology* 54: 837-844.

485 Price SA, SSB Hopkins, KK Smith, VL Roth. 2012. Tempo of trophic evolution and its impact on
486 mammalian diversification. *Proc. Natl. Acad. Sci* 109: 7008-7012.

487 Rasband WS. 1997. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA,
488 <https://imagej.nih.gov/ij/>, 1997-2018.

489 Rayfield EJ. 2005. Using finite-element analysis to investigate suture morphology: a case study using
490 large carnivorous dinosaurs. *Anatomical Record* 283A: 349-365.

491 Schluter D. 2000. The ecology of adaptive radiation. Oxford: Oxford University Press.

492 Sellers WI, Crompton RH. 2004. Using sensitivity analysis to validate the predictions of a
493 biomechanical model of bite forces. *Annals of Anatomy* 186: 89-95.

494 Shannon CE. 1948. A mathematical theory of communication. *The Bell System Technical Journal* 27:
495 379-423.

496 Svensson EI, RE Calsbeek. 2012. The adaptive landscape in evolutionary biology. Oxford: Oxford Univ.
497 Press.

498 Szarski, H. 1962. Some remarks on herbivorous lizards. *Evolution* 16: 529-529.

499 Taverne M, A-C Fabre, N King-Gillies, M Krajnovic, D Lisicic, L Martin, L Michal, D Petricioli, A
500 Stambuk, Z Tadic, C Vigliotti, B Wehrle, A Herrel. 2019. Diet variability among insular populations of
501 *Podarcis* lizards reveals diverse strategies to face resource-limited environments. *Ecology and*
502 *Evolution* 9: 12408-12420.

503 Taverne M, N King-Gillies, M Krajnovic, D Lisicic, Ó Mira, D Petricioli, I Sabolic, A Štambuk, Z Tadic, C
504 Vigliotti, B Wehrle, A Herrel. 2020. Proximate and ultimate drivers of variation in bite force in insular
505 lizards *Podarcis melisellensis* and *Podarcis siculus*. *Biological Journal of the Linnean Society* 131: 88-
506 108.

507 Taverne M, H Dutel, M Fagan, A Štambuk, D Lisicic, Z Tadic, A-C Fabre, A Herrel. 2021. From micro to
508 macroevolution: drivers of shape variation in an island radiation of *Podarcis* lizards in the Adriatic.
509 *Evolution* 75: 2685-2707.

510 Taverne M, T Decamps, O Mira, I Sabolic, JD da Silva, M Glogoski, D Lisicic, A Stambuk, A Herrel. 2022.
511 Relationships between dietary breadth and flexibility in jaw movement: a case study of two recently
512 diverged insular populations of *Podarcis* lizards. *Comparative Biochemistry and Physiology - Part A*
513 *Molecular & Integrative Physiology* 265: 111140

514 Vervust B, S Van Dongen, I Grbac, R Van Damme. 2009. The mystery of the missing toes: extreme
515 levels of natural mutilation in island lizard populations. *Functional Ecology* 23: 996-1003.

516 Wehrle BA, A Herrel, B-Q Nguyen-Phuc, S Maldonado Jr, RK Dang, R Agnihotri, Z Tadic, DP German.
517 2020. Rapid dietary shift in *Podarcis sicula* resulted in localized changes in gut function. *Physiological*
518 *and Biochemical Zoology* 93:396-415.

519

520 **Figure legends**

521 **Figure 1:** MDA model design illustrating the muscle bundles used of each muscle and their wrapping.
522 A: left lateral view of the skull, B: dorsal view, C: caudal view, D: ventral view. The jaw opener is
523 represented in yellow, the external adductors in shades of blue, the pseudotemporalis in red and
524 orange and the pterygoids in green. E: segments of the skull used to investigate the variation in stress
525 accumulation over the structure (here represented by a ventral view) in subsequent FEA. Note that
526 the bite points and the joints are indicated by white stars and white circles, respectively.

527 **Figure 2:** A: relationship between bite force (in Newtons), estimated by the MDA for the four models,
528 and the gape (in degrees). B: relationship between the conversion rate of the total muscle force into
529 bite force (in percentage) and the gape (in degrees) for all models. The two natural models (PK_PK:
530 including the morphology and the musculature of Pod Kopište, PM_PM: including the morphology
531 and the musculature of Pod Mrčaru) are represented by full lines, whereas the two hybrid models
532 (PK_PM: including the morphology of Pod Kopište and the musculature of Pod Mrčaru, PM_PK:
533 including the morphology of Pod Mrčaru and the musculature of Pod Kopište) are represented by
534 dashed lines.

535 **Figure 3:** Contribution (in percent) of the differences in musculature (in light gray) and skull shape (in
536 dark gray) in explaining the total difference in calculated bite force between individuals from the two
537 islands (Pod Kopište and Pod Mrčaru). The contributions were estimated for every gape tested (in
538 degrees) by comparing the change in calculated bite force induced by the change in musculature or
539 morphology alone (theoretical models) with the change in calculated bite force between the two
540 natural models.

541 **Figure 4:** von Mises stress distribution in the two natural models (Brown lines: PK_PK, green lines:
542 PM_PM) for the three gapes tested (full lines: 0°, dashed lines: 20°, dotted lines: 45°). Stresses are
543 plotted relative to the virtual skull section number (i.e. position along the antero-posterior axis for
544 which results were averaged; see methods). The left lateral, ventral, caudal and dorsal views of the

545 skull are represented (from left to right). Warmer colors are associated with higher von Mises stress
546 magnitudes (in MPa).

547 **Figure 5:** Consequences of skull shape change on the von Mises stress distribution. The graph
548 represents the mean advantage (in %) provided by the PM morphology along the skull (full line: 0°,
549 dashed line: 20°, dotted line: 45°). Here the models PK_PM and PM_PM (PK / Pod Kopište and PM /
550 Pod Mrčaru in the figure) were compared for each of the three gapes tested (see Figure S8 for the
551 alternative combination PM_PK vs. PK_PK). Hotter colors are associated with higher von Mises stress
552 magnitudes (in MPa).