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The contribution of functional traits to the understanding of palaeo-environmental changes

Léa Terray^{1*}, Emmanuelle Stoetzel², Anthony Herrel³, Raphaël Cornette¹

¹ Institut de Systématique, Evolution, Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, CP50, 57 rue Cuvier, 75005 Paris, France.

² HNHP, CNRS, Muséum National d'Histoire Naturelle, Sorbonne Université, UPD, Musée de l'Homme, Palais de Chaillot, 17 place du Trocadéro, 75016 Paris, France.

³ Mécanismes Adaptatifs et Evolution (MECADEV), Muséum national d'Histoire naturelle, CNRS, 55 rue Buffon, Paris, France

* Corresponding author (mail: lea.terray@mnhn.fr)

Abstract

Performance traits interact with the environment and are consequently relevant ecological indicators. In particular, performance traits implicated in feeding may be powerful ecological proxies as they are directly related to diet. However, performance traits have only rarely been used to better understand palaeo-environmental variation. In the present study we evaluate the usefulness of functional traits (i.e. estimated bite force) in reconstructing the palaeo-ecology of shrews. We [1] investigate the relationships between mandible morphology, bite force estimates, and the ecological context, and [2] assess the relevance of estimated bite force as a palaeo-environmental indicator. To do so, we use geometric morphometrics to quantify mandible shape diversity in shrews of the archaeological site El Harhoura 2 (Rabat, Morocco), dated from the Late Pleistocene to the Holocene. To avoid errors due to identification, morphological groups were used instead of taxa as units of diversity. In order to explore how phenotypic traits are linked to their environment, mandible shape and bite force estimates were compared with palaeo-environmental inferences for the El Harhoura 2 site extracted from the literature. As hypothesized in previous studies, morphological groups acted as phenotypic response units. Estimated bite force was related to palaeo-environmental variation over the considered period, with a particular sensibility to arid/humid environmental transitions. Functional characteristics of the morphological groups pointed out a potential change in climate that had not been evidenced by previous palaeo-environmental studies. The complementarity of morphological and functional indicators allowed us to infer functional convergence and divergence among shrews. Our results suggest that functional traits may be relevant indicators of changes in palaeo-environments. However, they do not exhibit characteristic responses to particular climatic transitions. This approach opens up new possibilities to explore the impact of environmental changes in extinct organisms.

1 **1. Introduction**

2 Performance traits, provide a direct link between ecology, morphology, and fitness (Arnold,
3 1983; Wainwright, 1994). They reflect the ability of individuals to perform ecologically
4 relevant tasks (Irschick *et al.*, 2008) and are subjected to selection (Irschick *et al.*, 2008). In
5 animals, performance traits are dependent on skeletal structures, variation in muscular
6 anatomy, contractile physiology, and variation in biomechanical traits such as lever arms.
7 Consequently, the relationship between morphological and functional traits is complex
8 (Wainwright, 1994; Irschick *et al.*, 2008). Different morphological traits can generate similar
9 functional outputs by redundancy (Alfaro, Bolnick, & Wainwright, 2005), which may lead to
10 functional convergence in organisms living in environments requiring similar performance
11 abilities (Wainwright, 2005; Young *et al.*, 2007, 2010). Conversely, one morphological trait
12 can affect different performance traits through trade-offs and facilitation. Trade-offs occur
13 when there is a conflicting demand on a phenotypic trait through its differential implication in
14 several performance traits (Garland & Losos, 1994; Van Damme *et al.*, 2003; Walker, 2007,
15 2010; Langerhans, 2009; Holzman *et al.*, 2011; Vanhooydonck *et al.*, 2011). Facilitation, on
16 the other hand, occurs when a similar demand is exerted on a phenotypic trait by several
17 different performance traits (Walker, 2007). Therefore, a complex "many-to-many"
18 morphology-performance relationship may exist (Bergmann & McElroy, 2014), implying that
19 morphological and functional traits may not respond similarly to changes in the environment.
20 Thus, functional traits may be relevant candidates to function as ecological indicators,
21 complementary to morphological traits.

22 Feeding is the function related to dietary ecology (Schwenk, 2000). Bite force is a
23 performance trait implicated in feeding. It is a performance trait directly linked to diet through
24 the mechanical demands imposed by variation in the mechanical properties of food items
25 (Anderson *et al.*, 2008; Herrel *et al.*, 2008; Dumont *et al.*, 2009; Santana *et al.*, 2010; Maestri
26 *et al.*, 2016). Changes in diet associated with bite force are considered one of the main
27 drivers of diversification in mammals (Christiansen & Wroe, 2007; Monteiro & Nogueira,
28 2011). Thus, bite forces may be a powerful ecological proxy and have been widely used to

29 characterize dietary ecology in vertebrates (e.g. Freeman, 1979; Losos, 1992; Herrel *et al.*,
30 2002; Huber, 2005; Kerr *et al.*, 2017).

31 Studies on bite force in fossil taxa are common as they may provide insights into the
32 behavioural ecology of extinct taxa (e.g. Erickson *et al.*, 1996; Therrien, 2005; Wroe *et al.*,
33 2005; Lappin *et al.*, 2017; Rinderknecht *et al.*, 2019). However, to our knowledge, estimates
34 of bite force have only rarely been applied to better understand palaeo-environmental
35 variation. Commonly, such inferences rely on community compositions and/or the
36 characterization of particular morphotypes (e.g. (Fernandez-Jalvo *et al.*, 1998; Stoetzel *et al.*,
37 2011; Comay, Weissbrod & Dayan, 2019; Royer *et al.*, 2020; López-García *et al.*, 2021).
38 These approaches associate a particular biological feature to a specific environment.
39 However, an organism's dietary ecology can be more accurately inferred from bite force than
40 by categorical ecological classifications (Santana *et al.*, 2010). Thus, bite force has a strong
41 potential to contribute meaningfully to understand paleo-environmental variation.

42 Functional aspects are also important determinants of morphological integration. This
43 concept defines the covariation patterns that exist between morphological traits (Badyaev &
44 Foresman, 2000, 2004; Young & Badyaev, 2006; Klingenberg, 2008; Klingenberg &
45 Marugán-Lobón, 2013). As a result of selection on performance, traits implicated in a same
46 function interact strongly, making them vary together (Klingenberg, 2008). This may be
47 particularly the case when they are linked through muscle insertions providing physical and
48 mechanical links between structures (Cheverud, Routman, & Irschick, 1997; Mezey,
49 Cheverud, & Wagner, 2000; Klingenberg, Mebus, & Auffray, 2003; Klingenberg, 2004). Thus,
50 changes in the strength of integration in the feeding apparatus could be related to changes in
51 bite force. Moreover, the less the modules in a structure like the mandible covary together
52 (i.e. the more modular they are), the more disparate are the organisms that can be produced
53 (Goswami *et al.*, 2014). Modularity patterns may consequently also be related to bite force
54 disparity. Moreover, changes in modularity are known to respond to environmental stresses
55 (Badyaev & Foresman, 2000). In the light of these facts, modularity in the feeding apparatus
56 may also be a relevant indicator for understanding variation in palaeo-environments.

57 The Moroccan archaeological site El Harhoura 2 (EH2) is located in the Rabat-Témara
58 region, on the North-Atlantic coast of Morocco. It is divided into 11 archeo-stratigraphical
59 levels covering a time period from the Late Pleistocene to the Holocene. Phenotypic
60 evolution related to environmental variation has been extensively studied at this site
61 (Stoetzel, 2009; Stoetzel *et al.*, 2010, 2011, 2012b; Stoetzel, 2017; Stoetzel *et al.*, 2017).
62 Small mammals such as rodents and shrews are a relevant model to consider palaeo-
63 climatic variation (Valenzuela *et al.*, 2009; McGuire, 2010; Escudé *et al.*, 2013; Verde
64 Arregoitia, Fisher & Schweizer, 2017). The ecological characteristics associated with species
65 (as diet, habitat, soil) allowed palaeo-climatic inferences at EH2 (e.g. Stoetzel *et al.*, 2011,
66 2013, 2017). Among the remains recovered at EH2, shrew mandibles have been given
67 peculiar attention (Cornette *et al.*, 2015a,c). Mandible shape is known for being highly plastic
68 and often varies with environmental conditions in these animals (Badyaev & Foresman,
69 2000; Caumul & Polly, 2005; Cornette *et al.*, 2015a,c; Souquet *et al.*, 2019).

70 In the present study, we sought to understand the relationship between the morphology
71 of the mandible in shrews, its modularity, and the estimated bite force over time. We further
72 explored whether a functional trait (bite force) can provide relevant information for the
73 understanding of palaeo-environments. Our approach is divided in three steps. 1) To
74 evaluate global morphological diversity, shrews were divided into morphological groups
75 (MGs). This characterization of biodiversity was chosen instead of species. MGs allow to
76 consider variations at several levels of diversity (inter- and intra-specific), which makes it a
77 relevant approach to characterize environment-related selection which acts at both levels
78 (Erwin, 2000; Lande, 2009; Boutin & Lane, 2014; Hautmann, 2020). We expected identified
79 MGs to be functional response units to changes in the environment, as has been suggested
80 in previous studies (Read *et al.*, 2014; Khare *et al.*, 2017). 2) Bite force was estimated
81 through the mechanical potential. Because performance and morphology are not strictly
82 related, selection can apply differently at both levels of organization (Irschick *et al.*, 2008).
83 Thus, quantifying variation in the mechanical potential may allow us to detect selection
84 events undetectable through morphological proxies. 3) Finally, variation in modularity was

85 characterized. An increase in modularity in shrew mandibles may allow morphological
86 diversification within a population utilizing the same resources irrespective of selection on
87 function (Young *et al.*, 2007, 2010) and as such be associated with variation in the
88 environment.

89

90 **2. Material and methods**

91 *2.1 El Harhoura 2 cave and data collection*

92 El Harhoura 2 cave (33°57'08.9" N / 6°55'32.5" W) is an archaeological site located on the
93 Moroccan Atlantic coast, a few km south of Rabat. The cave was occupied by Middle Stone
94 Age, Later Stone Age, and Neolithic human populations and has yielded numerous
95 archaeological material as well as the remains of large and small vertebrates (Nespoulet *et*
96 *al.*, 2008; El Hajraoui *et al.*, 2012; Stoetzel *et al.*, 2014). From top to bottom, the stratigraphy
97 of EH2 cave is structured in 11 layers. Eight of these levels are well dated and were included
98 in this study (L1, L2, L3, L4a, L5, L6, L7, L8) (Jacobs & Roberts, 2012; Ben Arous *et al.*,
99 2019, 2020). Palaeo-environmental data were deduced from faunal communities (Stoetzel,
100 2009; Stoetzel *et al.*, 2011, 2012b,a, 2014). An alternation of humid and arid periods has
101 been documented and the landscape was mainly dominated by steppes with an increase in
102 more wooded areas and water ponds during humid periods (**Figure 1**).

103 The material studied here is temporarily housed at the Muséum National d'Histoire
104 Naturelle, Paris, France. It was sampled during the 2005-2009 excavation campaigns of the
105 El Harhoura-Témara Archaeological Team (dir. R. Nespoulet and M.A. El Hajraoui). Four
106 species of white-toothed shrews are represented: the material is largely dominated by
107 *Crocidura russula* but *C. lusitania*, *C. tarfayensis* and *C. whitakeri* were also present
108 (Cornette *et al.*, 2015a,c). Among archaeological remains complete mandibles are rare and
109 most of the material is fragmented. Nevertheless, mandible fragments also carry relevant
110 morphological and palaeo-environmental information (Cornette *et al.*, 2015a,c) and can be
111 used to increase the sample. Here we used complete mandibles (Clpt) and three types of
112 mandible fragments (A, B, C). Chosen fragments are those whose shape best enables

113 species to be distinguished (Cornette *et al.*, 2015a). Fragmentation patterns are illustrated in
114 **Figure 2**. Extant material (Act) of the same four species from the Rabat area was added to
115 extend the time line to present day. The number of complete and fragmented mandibles
116 studied is indicated in **Table 1**. Data acquisition is described in Cornette *et al.* (2015a).

117

118 *2.2 Geometric morphometrics*

119 Two dimensional geometric morphometric analyses were used. All mandibles could not
120 be analyzed together because remains from various fragmentation patterns are not directly
121 comparable. We performed separate shape analyses for each fragment type (Cplt, A, B and
122 C) following the same protocol. Mandibles were analyzed through a landmark and sliding
123 semi-landmark based approach allowing the description of the shape of biological relevant
124 areas without anatomical landmarks (Bookstein, 1996; Zelditch, 2004; Gunz, Mitteroecker, &
125 Bookstein, 2005; Cornette *et al.*, 2013). Landmark locations for each fragment type are
126 indicated in **Figure 2**. Semi-landmarks were slid to minimise the bending energy.
127 Generalized Procrustes Analyses (GPA) were performed on each data set (Cplt, A, B and C)
128 to remove effects of translation, rotation, and scale and make objects comparable (Rohlf &
129 Slice, 1990). Resulting shape coordinates are the Procrustes residuals. These two last steps
130 were performed using the "gpagen" function of the "Geomorph" package (Adams & Otárola-
131 Castillo, 2013) in R version 3.6.3 (2020-02-29). To reduce data dimensionality, principal
132 component analyses were performed on Procrustes residuals for each data set (Cplt, A, B
133 and C) and we retained 95% of shape variation for the following analyses (Baylac & Frieß,
134 2005). All analyses were performed using R version 3.6.3 (2020-02-29).

135

136 *2.3 Morphological groups*

137 To assess shape diversity, mandibles were partitioned into morphological groups. First,
138 complete mandibles (Cplt) were clustered based on their shape. To do so a morphological K-
139 nearest neighbor method (KNN) was used. This method is adapted to small datasets, as is
140 the case here, because it is a non-parametric classification. In this algorithm, each shape

141 object is assigned to its nearest neighbor cluster. The "clues" function of the "clues" package
142 (Wang, Qiu & Zamar 2007) proposes an unsupervised KNN, meaning that the number of
143 clusters is inferred from the data itself, favoring the most robust partitioning of the dataset.
144 The robustness of the clusters is assessed by the Silhouette index (SI) (Kaufman &
145 Rousseeuw, 1990) which measures the strength of the clusters. SI is comprised between -1
146 and 1. The more SI is close to 1 (i.e. $SI > 0$), the more data points are correctly assigned to
147 their clusters, and conversely the more SI is close to -1 (i.e. $SI < 0$), the more data points are
148 mis-assigned (Wang *et al.*, 2007).

149 To visualize the morphological groups identified, the morphospace of complete
150 mandibles (Cplt) was plotted using the three first axes of the principal component analysis
151 computed on the Cplt data set. Deformations along axes were computed using the function
152 "PlotRefToTarget" from the "geomorph" package (Adams & Otárola-Castillo, 2013). Thin-
153 plate spline deformation grids representing differences between the extreme shapes of each
154 axis and the global mean shape of Cplt mandibles were generated.

155 Next, we tested the robustness of the morphological groups identified on complete
156 mandibles (Clpt) for each fragmentation pattern. Artificial A, B and C fragments were
157 computed from complete mandibles (Clpt) and strengths of the clusters based on fragments
158 were assessed using SI. This was achieved using the "get_Silhouette" function of the "clues"
159 package (Wang *et al.*, 2007).

160 Finally, the belonging of true A, B and C mandible fragments to the morphological groups
161 was determined *a posteriori* using the KNN classification algorithm with the "knn" function of
162 the "class" package (Venables & Ripley, 2002). Mean shapes of each morphological group
163 were computed from complete mandibles (Clpt) using the functions "mshape" and
164 "warpRefOutline" of the "Geomorph" package (Adams & Otárola-Castillo, 2013).

165

166 *2.4 Mechanical potential*

167 The mechanical potential as used in this study is the ratio of the muscle moment arm to
168 the jaw outlever. As such it is dependent on the geometry of the skull and mandible and the

169 insertion of the masticatory muscles (Herrel *et al.*, 2008; Chazeau *et al.*, 2013; Manhães,
170 Nogueira, & Monteiro, 2017; Ginot *et al.*, 2018, 2019). In particular, mandible shape is known
171 for being a good estimator of bite force (Brassard *et al.*, 2020a,b). As a proxy for overall
172 mechanical potential, we choose the mechanical potential of the temporalis muscle. This is
173 one of the main muscles involved in bite force generation in shrews (Herrel *et al.*, 2008;
174 Santana *et al.*, 2010; Brassard *et al.*, 2020a). It was estimated based on the moment arm of
175 the temporalis, computed from the mandible shape. The biomechanical model is presented
176 in **Figure 3**. It is defined as $MP = A / B$, where: A is the moment arm of the temporalis, B the
177 jaw out-lever, and MP the mechanical potential of the temporalis. MP was computed for
178 complete mandibles (Cplt) from Procrustes residuals.

179 However, MP does not reflect the phenotype in nature: because it is computed from
180 Procrustes residuals, it does not account for size variation in the data set, nor for
181 compensatory effects of musculature. Moreover, it only considers a single force - the
182 temporalis muscle - which is applied uniformly (Young *et al.*, 2007). All these parameters are
183 important drivers of the mechanical potential, in particular size (Wroe *et al.*, 2005; Freeman &
184 Lemen, 2008; Herrel *et al.*, 2008; Chazeau *et al.*, 2013; Manhães *et al.*, 2017; Ginot *et al.*,
185 2018, 2019; Brassard *et al.*, 2020a). In order to increase the accuracy of our estimate of bite
186 force, we corrected MP to take size into account.

187 It is known that MP is linearly correlated to mandible/skull size in some mammals,
188 including shrews (Nogueira, Peracchi, & Monteiro, 2009; Cornette *et al.*, 2015b; Manhães *et al.*
189 *et al.*, 2017; Ginot *et al.*, 2018; Brassard *et al.*, 2020a). As MP is computed from mandible
190 shape, this means that it has an allometric component, i.e. even if MP is size-free, a part of
191 the MP results from the influence of size. To confirm this assumption in our data set, we
192 tested it on complete mandibles (Cplt) by performing a regression of the log-transformed
193 centroid size on the log-transformed MP using the “lm” function of the “stats” package.
194 Centroid size (Csize) is a size estimator widely used in geometric morphometrics. It is
195 defined as the square root of squared distances of all landmarks of a mandible from its
196 centroid (Klingenberg, 2016).

197 Corrected MP (cMP) was subsequently expressed as: $cMP = MP + f(\text{size})$ **(1)**, where MP
198 is the mechanical potential and $f(\text{size})$ the part of cMP due to size. It is also known that \log_{10}
199 bite force is linearly correlated to size (Wroe *et al.*, 2005; Chazeau *et al.*, 2013; Manhães *et*
200 *al.*, 2017; Ginot *et al.*, 2019; Brassard *et al.*, 2020a). Thus, for cMP to be a good estimator of
201 bite force, log-transformed cMP must also be linearly correlated to size. So, in **(1)** we have
202 $f(\text{size}) = a \cdot \text{size} + b$ **(2)**. The objective here is to determine a and b .

203 We assume that the allometric part of MP is proportional to the influence of size on MP.
204 Thus, a and b of **(2)** can be found by performing a linear regression of size on MP. However,
205 our data have a temporal component that we need to consider, otherwise, we may lose part
206 of this temporal information as MP and size are both related to time. We performed a
207 multivariate regression of size and time on MP. Log-transformed values of MP and Csize
208 were used. a and b of **(2)** were defined with the parameters of this regression: a as the
209 coefficient of $\log(\text{Csize})$ and b as the intercept. We obtained: $\log(cMP) = \log(MP) +$
210 $a \cdot \log(\text{Csize}) + b$ **(3)**.

211 To evaluate whether cMP is a good estimator of bite force, we assessed its reliability on a
212 dataset of simulated data:

213 1. 1000 bite forces and associated sizes were simulated. Values were randomly
214 generated according to a normal distribution using the "rnorm" function of the "stats"
215 package, with for bite forces the constraint of a mean of 0.3 and a standard deviation of 0.15,
216 and for size a mean of 3500 and a standard deviation of 500. Those constraints aimed to
217 generate a dataset as close as possible to what is observed for the species present in our
218 data set.

219 2. MP was computed using **(3)**, where cMP was replaced by bite force values and with a
220 and b arbitrarily fixed (to represent the "real" relation between MP, bite force and size in the
221 simulated dataset). 30 couples of a and b were randomly generated using the "rnorm"
222 function of the "stats" package and tested. They were computed in order to be similar to the
223 a and b found previously. As the correlation between \log_{10} bite force and size is strictly
224 positive (Wroe *et al.*, 2005; Chazeau *et al.*, 2013; Manhães *et al.*, 2017; Ginot *et al.*, 2019;

225 Brassard *et al.*, 2020a), *a* should be strictly positive and was generated with the constraint of
226 a mean of 3 and a standard deviation of 3. *b* was generated with the constraint of a mean of
227 0 and a standard deviation of 5, to test positive and negative values. Tested couples of *a* and
228 *b* are presented in **Table S1**. To simulate measurement error, Gaussian noise was added to
229 computed MP (error tested at 0.01, 0.05 and 0.1).

230 3. cMP were calculated using **(3)**, with *a* and *b* found previously.

231 4. For each pair of values in the simulated dataset, we tested whether the relation
232 between the bite force of individuals was respected by cMPs (for example, if we have two
233 individuals X and Y such that: bite force X > bite force Y, we must also have cMP X > cMP
234 Y). Thus, variation in cMP reflects, in a relative way, variation in bite force. Mean, min and
235 max scores of reliability among the 30 *a* and *b* couples were computed for the three
236 measurement error estimates.

237 To obtain the cMP of fragmented mandibles (A, B and C) we predicted it from the cMP of
238 complete ones (Cplt) according to the following protocol. First, to evaluate the reliability of the
239 prediction, we performed covariation analyses between artificial fragments generated from
240 the complete mandibles (Cplt) corresponding to fragmentation patterns (A, B and C) and
241 cMP. We used two-block partial least squares analyses (PLS) which assesses the
242 covariance between two sets of variables. This step was done using the "two.b.pls" function
243 of the "geomorph" package (Adams & Otárola-Castillo, 2013), which performs a PLS
244 adapted to shape data. Then, cMP of fragmented mandible was predicted using the "pls" function of the "pls" package (Mevik & Wehrens, 2007). This function allows prediction based
245 on the covariation between the two variables.

247 Shapes associated with the strongest and weakest cMP were computed. Those shapes
248 were estimated based on the linear regression of cMP on shapes of complete mandibles
249 (Cplt) using "procD.lm" of the "Geomorph" package (Adams & Otárola-Castillo, 2013). They
250 were then computed with the "warpRefOutline" function of the same package.

251 Changes of cMP through time were investigated in two ways:

252 1) global cMP per layer. Differences between layers were tested through pairwise
253 comparisons testing using the "pairwise.t.test" function of the "stats" package which correct
254 for multiple testing.

255 2) cMP per morphological group per layer.

256 For both, standard deviation was computed on each layer as a measure of variance.

257

258 2.5 Modularity

259 As the abundance of complete mandibles (Clpt) per layer was not sufficient, the
260 modularity analysis was performed based on the C fragmentation pattern on complete (Clpt)
261 and C fragments. We divided the mandible into two modules *a priori* according to the
262 hypothesized primary developmental modules defined in the literature (Cheverud *et al.*,
263 1997; Mezey *et al.*, 2000; Klingenberg *et al.*, 2003; Klingenberg, 2004) (**Figure 3**).

264 To quantify the modular structure of the mandible, we computed the Covariance Ratio
265 (CR) per layer. CR compares the global covariation between hypothesized modules relatively
266 to the covariation within those modules (Adams, 2016). The modularity hypothesis
267 (independence of the hypothesized modules) is verified when $CR < 1$. This measure is
268 unaffected by sample size or the number of variables (Adams, 2016). It was performed using
269 the "modularity.test" function of the "geomorph" package (Adams & Otárola-Castillo, 2013).

270

271 3. Results

272 3.1 Morphological groups

273 Three morphological groups were detected among complete mandibles (Clpt). We
274 obtained an $SI=0.15$, meaning that the morphological partitions obtained with the clustering
275 are correct. This partitioning was also valid for A, B, and C fragments (A fragments: $SI=0.1$; B
276 fragments: $SI=0.09$; C fragments: $SI=0.1$).

277 A visualization of the morphological groups on the principal component analysis of
278 complete mandibles (Cplt) and their deformations along major axes is presented in **Figure 4**.
279 Mean shapes of the morphological groups are presented in **Figure 5**. The first group

280 (illustrated in grey in **Figure 5**) has an elongated mandibular body, a more anteriorly inclined
281 coronoid, and a more dorsally oriented condylar process compared to the average mandible
282 shape. The second group (illustrated in red in **Figure 5**) displays a shape similar to the mean
283 shape. The third group (illustrated in blue in **Figure 5**) has a short mandibular body, a
284 coronoid process that is slightly more posteriorly inclined, and a condylar process that is
285 more ventrally oriented.

286

287 *3.2 Mechanical potential*

288 Log-transformed MP revealed to be weakly and negatively correlated to log-transformed
289 Csize ($R^2=0.014$, $P=0.0029^{**}$, 52 degrees of freedom). The multivariate regression (**3**) was
290 also significant ($R^2=0.12$, $P=0.023^*$, 50 degrees of freedom). We obtained $a=0.30$ (coefficient
291 of log-transformed Csize) and $b=-3.47$ (intercept). The reliability scores of cMP based on the
292 simulated data are indicated in **Table 2**.

293 The PLS results showed that cMP is well related to A, B, and C fragments generated
294 from complete mandibles (Clpt) (PLS: A fragments, $r\text{-pls}=0.66$, $P<0.05$; B fragments, $r\text{-}$
295 $\text{pls}=0.75$, $P<0.05$; C fragments, $r\text{-pls}=0.64$, $P<0.05$), ensuring the reliability of predicted cMPs
296 for fragments.

297 Mean shapes associated with the strongest and weakest cMP are presented in **Figure 6**.
298 The shape associated with the weakest cMP (left in **Figure 6**) is characterized by an
299 elongated mandibular body and a short mandibular branch compared to the global average
300 shape of the mandible. On the contrary, the shape associated with the strongest cMP (right
301 in **Figure 6**) displays a short mandibular body and a long mandibular branch.

302 Global changes in cMP through time are presented in **Figure 7A**. In L7, a period
303 characterized by an open and arid environment, a great variability in cMPs co-exist
304 compared to other layers. An important increase in average cMP is observed from L7 to L5
305 and then cMP decreases until present resulting in cMP values similar to those observed in
306 L7. However, pairwise testing indicates that only the cMP of L5-L7 and Act-L5 are
307 significantly different ($P<0.05$).

308 Changes in cMP per morphological group through time are presented in **Figure 7B**. Not
309 all morphological groups show the same variation in cMP over time, nor the same degree of
310 variability. However, the three groups display a higher variability in L7. Overall, one of the
311 morphological groups (indicated in blue in **Figure 7B**) has a higher cMP than the others. The
312 two other groups (indicated in red and black in **Figure 7B**) display similar cMP values. In L5
313 the cMP of the three morphological groups converges toward high cMP values. Then,
314 starting from L5, there is a drop of mandible shape diversity with the disappearance of one
315 morphological group. In L4a and L3, the two remaining groups show a divergence in cMP
316 towards respectively lower and higher values. In L2 a second diversity drop occurs with the
317 disappearance of another group. From then onwards only one morphological group is
318 consistently present, the two others show only few occurrences.

319

320 *3.3 Modularity*

321 Changes in CR over time are presented in **Figure 7C**. CR was not statistically significant
322 in L2 and L4a. There are two main changes in CR values, first a slight increase in L7
323 (CR=0.86, P<0.05) followed by a strong increase from L5 to L3, with a peak in L3 (CR=0.95,
324 P<0.05).

325

326 **4. Discussion**

327 In this study, we aimed [1] to investigate the relationship between shrew mandible
328 morphology, modularity, and estimated biting forces (cMP) over time and [2] to evaluate
329 whether a performance trait (bite force) could provide relevant information in the
330 understanding of palaeo-environments. To do so, we explored mandible shape diversity in
331 archaeological shrews, and computed the covariation ratio and estimated mechanical
332 potential over time. First, we discuss the meaning of morphological groups and the benefits
333 of this approach. Next, we focus on the relation between mandible shape and mechanical
334 potential. Finally, we address the variation in these traits over time and compare them to

335 environmental inferences from the literature to assess their potential relevance for paleo-
336 environmental studies.

337

338 4.1 Morphological groups

339 Over the considered period, up to three morphological groups of mandibles were found
340 among the four shrews species present at EH2 (*Crocidura russula*, *C. whitakeri*, *C.*
341 *tarfayensis* and *C. lusitania*) (Cornette *et al.*, 2015a,c). The assessed morphological diversity
342 is consequently above the species level. Simplification of information is a risk of the
343 morphological group approach (Read *et al.*, 2014), but it provides access to a partition of
344 variability that is potentially more informative ecologically than taxonomically when the
345 studied biological object is sensitive to environmental change (Alperin, Cusminsky, &
346 Bernasconi, 2011; Read *et al.*, 2014; Khare *et al.*, 2017), which is the case for shrew
347 mandibles (Badyaev & Foresman, 2000; Young *et al.*, 2010). The morphological groups
348 reveal variations in the number of morphotypes independent of the number of species. For
349 example, in the recent layers of EH2 (from L4a until today) only one or two morphological
350 groups are present per layer whereas all four species are still present suggesting
351 morphological convergence across these species (Cornette *et al.*, 2015c). The morphological
352 groups are characterized by different functional outputs and in their response to
353 environmental changes. Except in L5, they display a different cMP and show different trends
354 over time, especially from L4a and L3. To sum up, morphological groups are characterized
355 by differences in morphology, functional output, and their response to environmental change.
356 Thus, they represent morphological and functional response units to external variation.

357 Nevertheless, because we used only three of the five types of mandible fragments that
358 were used in Cornette *et al.* (2015a, c), we reduced the initial sampling which can result in a
359 potential loss of diversity. However, unused fragments were the least informative and reliable
360 and could have introduced uncertainties into the results of this study which is why we chose
361 to exclude them.

362

363 *4.2 Mandible shape and mechanical potential*

364 The relation between rostrum elongation and mechanical potential is intuitive as a longer
365 rostrum increases the jaw out-lever and consequently results in a lower mechanical potential.
366 This is illustrated by the negative correlation between MP and mandible size. This type of
367 relation between MP and mandible size has also been observed in other mammals (e.g.
368 Casanovas-Vilar & van Dam (2013) for squirrels, Nancy (1982) for felids). This implies that
369 the smallest specimens have a higher mechanical potential than the largest ones.
370 Mechanical potential and size are both important drivers of bite force. These results suggest
371 that the relative importance of these drivers varies between small and large specimens.
372 Conformations may be a more important driver of bite force than size in small specimens
373 compared to large ones. Nevertheless, the weakness of this correlation suggest that the
374 specimens used here are rather uniform in size.

375 Concerning the mandibular branch, our results are consistent with Young *et al.* (2007)
376 who found that a high mechanical potential was associated with a greater distance between
377 the condylar and the coronoid processes. This is related to muscles of the masticatory
378 apparatus: the coronoid process is the place of insertion of the temporalis muscle, the
379 condylar process of the external pterygoid and the angular process of the internal pterygoid
380 and the masseter. They all participate in generating bite force and impact bone shape in
381 shrews (Furió *et al.*, 2010; Cornette *et al.*, 2015c).

382 In the light of the previous, the mean shapes of morphological groups display
383 morphological features that can be related to their mechanical potential. The group
384 displaying the highest mechanical potential (in blue on **Figure 6** and **Figure 7**) is the one
385 with the shortest mandibular body and the closest condylar and coronoid processes, which
386 are morphological and functional particularities related to hard diet specialists in shrews
387 (Young *et al.*, 2007). Conversely the group with the weakest mechanical potential (in grey
388 **Figure 6** and in black on **Figure 7**) displays features characteristic of soft diet specialists in
389 shrews (Young *et al.*, 2007). The third group (in red on **Figure 6** and **Figure 7**) showing

390 average features likely regroups generalist shrews. Thus, morphological groups appear to
391 highlight ecological specialisations.

392 Modularity might be a key concept to understand the link between morphological and
393 functional variation. Three main decreases in modularity (i.e. increases in CR) are observed
394 in the sequence at EH2 in L7, L5, and L3. In L7, the three morphological groups display
395 unusual variability in cMP (it is important to note that this is the layer with the largest sample).
396 In L5, the three morphological groups show remarkably similar cMPs, meaning similar
397 functional outputs. Finally, in L3, the two morphological groups display highly divergent
398 functional outputs. Each decrease in modularity (i.e., increase in CR) in the mandible is
399 associated with an increase in the ability of a form to produce more diverse functional
400 outputs, allowing either divergence (as in L7 and L3) or convergence (as in L5) in the cMP.
401 These results are in contradiction with Young *et al.* (2010) and Young *et al.* (2007), who
402 found that extensive modularity allowed shrews with more diverse morphologies to produce a
403 similar functional output. However, Young *et al.* (2010) underlined that adaptive responses
404 are highly variable, even at a population level, which may explain the difference observed in
405 comparison to our results. Maybe the adaptive strategy here implies a different relation
406 between mechanical potential and the modularity of the mandible. A possibility might involve
407 variation in skull shape which was not studied here (Cornette *et al.*, 2015c).

408

409 *4.3 Mechanical potential as a palaeo-environmental indicator*

410 When considering both global and per morphological group changes in cMP, four
411 important functional variations were detected over time in L7, in L5, during L4a-L3 and in L2.
412 Three of these (in L7, L5 and L2) match transitions from humid to arid environments
413 (Stoetzel, 2009; El Hajraoui *et al.*, 2012).

414 In L7 we observed a high morphological diversity (with the presence of the three
415 morphological groups), the co-existence of highly diverse cMPs (**Figure 7A and B**) and a
416 decrease in modularity (**Figure 7C**). An increase in the diversity in cMP could be caused by a
417 release of selective pressures on this trait, allowing shrews with diverse abilities to survive.

418 This could be due to the availability of more diverse resources in the environment of L7 than
419 in those of other layers. However, the environment in L7 is characterized by an open and dry
420 environment of arid steppes, and seems to present less ecological diversity than some other
421 layers (Stoetzel, 2009; El Hajraoui *et al.*, 2012). Another hypothesis might be that a lack of
422 resources drives character displacement in shrews with forms becoming highly specialized in
423 the consumption of different resources. Moreover, the masseter and the medial pterygoid
424 muscles also participate in generating bite force (Herrel *et al.*, 2008; Santana *et al.*, 2010;
425 Brassard *et al.*, 2020b,a). Yet, these muscles are implicated in the consumption of different
426 types of resources, as in bats where the masseter allows the consumption of more soft
427 resources (Santana *et al.*, 2010). Moreover, functional variation is accompanied by a
428 decrease in modularity. Variation in the degree of covariation between mandibular modules
429 in shrews may be related to stressful environmental conditions (Badyaev & Foresman, 2000,
430 p. 200). In the light of this fact, it is more likely that during the period covered by L7, shrews
431 endured particularly stringent environmental conditions, with fewer and/or different available
432 resources than before. As shrews are opportunistic, they might have switched their diet
433 during this period inducing a release on the functioning of the temporalis muscle. However,
434 as L7 is the layer with the largest sample this may bias our observations.

435 In L5, the cMP of two morphological groups greatly increases and the cMP of the three
436 groups converges toward high values (**Figure 7A and B**). Pairwise testing indicates that
437 global cMP is significantly different in L5 compared to L7 and present day (**Figure 7A**), and
438 consequently supports the hypothesis of a cMP convergence towards high values in L5. This
439 functional convergence is not associated with a convergence of the morphological groups,
440 which is not surprising as those two types of convergence (functional and morphological) can
441 be independent (Stayton, 2006). The group of soft diet shrews (in black on **Figure 7B**) is
442 possibly subjected to a selection causing large sized specimens to produce the highest
443 mechanical potential (as illustrated in **Figure S1**). Here we have a case of functional
444 redundancy: three distinct morphologies producing similar functional outputs (Alfaro *et al.*,
445 2005). This is observed in environmental conditions requiring similar performance abilities:

446 distinct morphologies are then able to adapt to similar functional demands (Wainwright,
447 2005; Young *et al.*, 2007, 2010). This functional convergence could be explained by an
448 increase in the selective pressures caused by fewer or different resources compared to
449 previous layers, as the transition to L5 is towards a more arid environment (Stoetzel, 2009; El
450 Hajraoui *et al.*, 2012).

451 In L4a, the morphological group of soft diet shrews is not present anymore. It must have
452 disappeared during the previous arid period during which soft resources may have been
453 scarce. It is known that the insect cuticle becomes harder under dryer environments (Klocke
454 & Schmitz, 2011). The cMP of the two remaining groups diverged distinctly. The supposed
455 strong selection pressure(s) leading previously to the functional convergence in L5 must
456 have eased. This is congruent with existing environmental inferences, as the environment in
457 L4a is hypothesized to be very similar with conditions in L6 (Stoetzel, 2009; El Hajraoui *et al.*,
458 2012). In L3, the divergence between cMP of the two groups increases. We can hypothesize
459 that those two shrew morphotypes were subject to functional divergence. Each group of
460 shrews may have specialized in the acquisition of a different food resource. The group
461 displaying high cMP specializing in hard, large objects, requiring mechanical potential with an
462 important contribution from the temporalis, and the other group specializing on softer, smaller
463 items, which requires less contribution of the temporalis. This divergence might be caused by
464 competition occurring among shrews. Such a competition has been suggested between
465 *Crocidura russula* and other shrews species at EH2 at the exact same period (Cornette *et al.*,
466 2015c), and shrew dietary specialization might be a response to competition (Smith &
467 Remington, 1996). In L2, a second diversity drop is observed with the disappearance of the
468 group of hard diet specialists. As in L5, this event occurred following an arid period during
469 which less diverse resources may have been available. Only the group of generalist shrews
470 is continuously present up to present.

471 Interestingly, extant shrews display a particularly weak cMP, without important
472 environmental changes. Once again, this might be linked to a release of selective pressures
473 on the temporalis resulting from a switch in diet (Santana *et al.*, 2010). Another explanation

474 might be a selection towards weaker cMP. This is counter-intuitive, however, as selection for
475 lower performance likely only occurs when the trait is energetically expensive to maintain or
476 involved in trade-offs with other more relevant trait (Irschick *et al.*, 2008). The energy
477 previously allocated to the mechanical potential of the temporalis might have been
478 reallocated to another performance trait under stronger selection in the novel environment.

479

480 **5. Conclusion**

481 To conclude, results of the present study illustrate the relevance of functional traits to
482 detect palaeo-environmental transitions. Estimated bite forces showed variation in relation to
483 palaeo-environmental changes over the considered period (Late Pleistocene - Holocene),
484 with a particular sensibility to transition between arid and humid environments. The
485 complementary nature of morphological and functional indicators allowed to infer and discuss
486 the possible evolutionary and ecological processes involved. Functional traits have a great
487 potential for refining paleo-environmental and palaeo-ecological inferences. Moreover, they
488 appear to be relevant indicators palaeo-environmental transitions and offer a range of
489 opportunities to explore the impact of environmental changes on extinct organisms.

490

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502

503 **Conflict of interest**

504 The authors declare that they have no conflict of interest.

505

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797 **Figure captions**

798 **Figure 1:** Stratigraphy, datation and characteristic environments of the eight layers of EH2
799 used in this study (Stoetzel, 2009; Jacobs *et al.*, 2012; Janati-Idrissi *et al.*, 2012; Nespoulet &
800 El Hajraoui, 2012; Stoetzel *et al.*, 2012a; Ben Arous *et al.*, 2019, 2020). Three datations
801 methods were used: AMS-14C (based on organic remains), US-ESR (Combined uranium
802 series and Electron Spin Resonance) and OSL (Optical Stimulated Luminescence). Light
803 blue layers: humid and open environment; dark blue layers: humid and close environment;
804 light yellow layers: arid and open environment; dark yellow layers: arid and semi-open
805 environment. Unused layers in this study are in grey.

806 **Figure 2:** Types of fragments and their landmark (red points) and semi-landmark (blue
807 points) locations. Clpt: complete mandibles; A: fragmented mandibles of type A; B:
808 fragmented mandibles of type B; C: fragmented mandibles of type C.

809 **Figure 3:** Biomechanical model used to estimate the mechanical potential of the temporalis
810 muscle (MP) from mandible shape. V is the vector that starts at mid distance between
811 landmarks 3 and 5 and has for direction the intersection between the line passing through
812 landmarks 3 and 4 and the parallel of the line passing through landmarks 2 and 3 passing
813 through 5. A is the moment arm of the temporalis (the distance between the landmark 2 and
814 the vector). B is the jaw outlever (the distance between landmarks 1 and 2). $M1$ and $M2$ are
815 the definitions of the two hypothesized modules of the mandible from the literature (Cheverud
816 *et al.*, 1997; Mezey *et al.*, 2000; Klingenberg *et al.*, 2003; Klingenberg, 2004) and landmarks
817 implicated (C fragmentation pattern). $M1$: ascending ramus module; $M2$: alveolar region
818 module.

819 **Figure 4:** Principal component analysis on complete mandibles (Cplt).

820 **Figure 5:** Mean shapes of the three morphological groups (in grey, red and blue) compared
821 to the global mean shape (in black) of complete mandibles (Cplt).

822 **Figure 6:** Mean shapes associated with the strongest (right) and weakest (left) cMP (in
823 yellow) compared to the global mean shape (in black) of complete mandibles (Cplt). Strength
824 of the cMP is symbolized by the size of the shrew drawing under the models.

825 **Figure 7:** Global changes in cMP (A), cMP per morphological groups (B) and CR (C) of the
826 shrews of EH2, from L8 until current day. Environmental conditions (Stoetzel, 2009; El
827 Hajraoui *et al.*, 2012) are indicated by background colors. For cMP, standard deviation is
828 indicated for each point. A: layers displaying significant different cMP are indicated in red. B:
829 Black is the first morphological group; red is the second morphological group; blue is the third
830 morphological group. C: Dotted lines indicate missing values.

831 **Table 1:** Abundance of remains studied (complete and fragmented mandibles).

Layer	Clpt	A	B	C	TOTAL
Act	16	-	-	-	16
L1	2	1	-	2	5
L2	2	4	1	2	9
L3	3	1	1	1	6
L4a	2	1	-	1	4
L5	10	-	2	3	15
L6	10	2	4	9	25
L7	9	7	13	10	39
L8	-	6	10	11	27
TOTAL					146

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834 **Table 2:** Reliability scores of cMP for the three tested measure errors.

Measure error	0.01	0.05	0.1
Mean	82%	82%	81%
Max	98%	96%	93%
Min	66%	66%	66%

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837 **SUPPORTING INFORMATION**

838 **Table S1:** Couples of *a* and *b* tested on the simulated data set of bite forces and cMPs to
 839 assess the reliability of cMP as an estimator of bite force.

<i>a</i>	<i>b</i>
3.64	0.87
6.97	9.11
2.30	4.38
1.60	2.63
5.74	-2.40
8.56	-7.38
0.89	-1.81
7.09	5.49
1.89	-1.52
1.35	5.30
0.42	2.27
4.44	1.08
2.21	4.32
2.01	-6.27
5.74	-1.78
0.92	3.07
4.55	-5.31
3.34	0.82
5.90	0.86
7.90	4.29
0.40	-3.71
1.64	-7.20
1.03	9.24

0.38	-2.87
2.82	-4.73
4.60	-3.80
1.64	1.13
4.73	2.03
5.78	1.55
5.58	-0.37

840

841 **Figure S1:** Global changes in Csize (A) and Csize per morphological groups (B) of the
842 shrews of EH2, from L8 until current day. Environmental conditions (Stoetzel, 2009; El
843 Hajraoui *et al.*, 2012) are indicated by background colors. Standard deviation is indicated for
844 each point. B: Black is the first morphological group; red is the second morphological group;
845 blue is the third morphological group.

846 **Figure S2:** Global changes in the moment arm of the temporalis (A on Figure 3) (A) and
847 lever arm of the temporalis per morphological groups (B) of the shrews of EH2, from L8 until
848 current day. Environmental conditions (Stoetzel, 2009; El Hajraoui *et al.*, 2012) are indicated
849 by background colors. Standard deviation is indicated for each point. B: Black is the first
850 morphological group; red is the second morphological group; blue is the third morphological
851 group.