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

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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

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

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

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

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

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

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

2. Material and methods

2.1 El Harhoura 2 cave and data collection

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

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

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

2.2 Geometric morphometrics

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

2.3 Morphological groups

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

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

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

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

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

2.4 Mechanical potential

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

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

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

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

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

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

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

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

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

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

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

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

To obtain the cMP of fragmented mandibles (A, B and C) we predicted it from the cMP of complete ones (Cplt) according to the following protocol. First, to evaluate the reliability of the prediction, we performed covariation analyses between artificial fragments generated from the complete mandibles (Cplt) corresponding to fragmentation patterns (A, B and C) and cMP. We used two-block partial least squares analyses (PLS) which assesses the covariance between two sets of variables. This step was done using the "two.b.pls" function of the "geomorph" package (Adams & Otárola-Castillo, 2013), which performs a PLS 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 on the covariation between the two variables.

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

Changes of cMP through time were investigated in two ways:

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

2) cMP per morphological group per layer.

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

2.5 Modularity

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

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

3. Results

3.1 Morphological groups

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

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

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

3.2 Mechanical potential

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

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

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

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

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

3.3 Modularity

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

4. Discussion

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

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

4.1 Morphological groups

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

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

4.2 Mandible shape and mechanical potential

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

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

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

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

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

4.3 Mechanical potential as a palaeo-environmental indicator

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

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

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

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

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

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

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

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

5. Conclusion

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

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure captions

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

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

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

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

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

Figure 6: Mean shapes associated with the strongest (right) and weakest (left) cMP (in yellow) compared to the global mean shape (in black) of complete mandibles (Cplt). Strength 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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833

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.