Divergent in shape and convergent in function: adaptive evolution of the mandible in Sub-Antarctic mice

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Abstract

Convergent evolution in similar environments constitutes strong evidence of adaptive evolution. Transported with people around the world, house mice colonized even remote areas, such as Sub-Antarctic islands. There, they returned to a feral way of life, shifting towards a diet enriched in terrestrial macroinvertebrates.

Here, we test the hypothesis that this triggered convergent evolution of the mandible, a morphological character involved in food consumption. Mandible shape from four Sub-Antarctic islands was compared to phylogeny, tracing the history of colonization, and climatic conditions.

Mandible shape was primarily influenced by phylogenetic history, thus discarding the hypothesis of convergent evolution.

The biomechanical properties of the jaw were then investigated. Incisor in-lever and temporalis out-lever suggested an increase in the velocity of incisor biting, in agreement with observations on various carnivorous and insectivorous rodents. The mechanical advantage related to incisor biting also revealed an increased functional performance in Sub-Antarctic populations, and appears to be an adaptation to catch prey more efficiently. The amount of change involved was larger than expected for a plastic response, suggesting microevolutionary processes were evolved.

This study thus denotes some degree of adaptive convergent evolution related to changes in habitat-related changes in dietary items in Sub-Antarctic mice, but only regarding simple, functionally relevant aspects of mandible morphology.

Keywords

Mus musculus domesticus; geometric morphometrics; adaptive convergence; mouse mandible; biomechanics

Introduction

Convergent evolution in response to similar environments constitutes one of the most convincing lines of evidence for adaptive evolution (Harmon et al. 2005). It has been shown in traits as diverse as limbs in lizards (Losos et al. 1997), plates, pelvis shape and oral jaws in fishes (Albertson et al. 2003; Shapiro et al. 2006; Marchinko and Schluter 2007), and head morphology in snakes (Aubret and Shine 2009). However, similar functional performance can be achieved by different shapes (Wainwright et al. 2005). Regarding complex traits, convergent adaptation may thus be obscured by the fact that only some aspects will be functionally relevant and hence prone to convergent evolution.

The house mouse (*Mus musculus domesticus*) is a highly successful global invader (Lowe et al. 2000). Being commensal, it followed the movement of people around the world and, consequently, is now present on four continents. It colonized even remote and inhospitable environments, such as Sub-Antarctic islands. On these remote islands, the mice face considerable environmental stresses (Berry et al. 1978), with conditions widely departing from their usual commensal habits. These result in strong selective pressures for adapting to the local environments that could trigger convergent evolution. Among the traits that might be under selection, those related to food exploitation would have been important for the survival of the colonizing individuals. Mice shifted their diet from their usual omnivorous-granivorous diet to a larger proportion of terrestrial animal prey, mostly above-ground and litter macroinvertebrates in various Sub-Antarctic islands (Gleeson and Van Rensburg 1982; Copson 1986; Rowe-Rowe et al. 1989; Chown and Smith 1993; Le Roux et al. 2002; Smith et al. 2002; van Aarde and Jackson 2007). An associated change in mandible shape was documented in mice from the small Guillou Island within the Kerguelen archipelago (Renaud et al. 2013), which provided a functional advantage in biomechanical ratios (Renaud et al. 2015) and was interpreted as an adaptive response to the dietary change of the mice on Guillou Island. It is thus a strong candidate to test convergent morphological evolution in mouse populations that colonized different Sub-Antarctic islands.

Here, the mandible shape of the house mice was thus quantified using 2D geometric morphometrics for specimens coming from three remote Sub-Antarctic areas: Falklands, Marion Island, and the Kerguelen archipelago (Fig. 1). As shown by phylogenetic data, colonization of the different islands occurred independently, from different sources populations (Hardouin et al. 2010). Even on the Kerguelen archipelago, two independent colonization events occurred, with two islets having a different phylogenetic signature than the rest of the archipelago (Hardouin et al. 2010). Guillou

Island is inhabited by the most common Kerguelen haplogroup, and Cochons Island by the second, more restricted haplogroup. The functional performance of the mandible shape was assessed using out-lever arms describing incisor and molar biting; and out-lever arms approximating the action of the main masticatory muscles. Biomechanical ratios (Anderson et al. 2014) were derived from these in- and out-levers, which were also described as a landmark configuration using geometric morphometrics. A phylogenetic study based on the mitochondrial D-loop and nuclear microsatellites provided a background about the colonization history of each insular population. The objectives of this study were thus: (1) Can convergent morphological evolution be evidenced on the different Sub-Antarctic islands; and (2) Is the convergent evolution more evident in functionally relevant traits than on the overall jaw shape?

Material and Methods

Material

Four Sub-Antarctic islands were sampled (Fig. 1). Two small islands from the Kerguelen Archipelago (Sub-Antarctic Indian Ocean) were considered, corresponding to different waves of colonization and thus having a distinct genetic signature (Hardouin et al. 2010): Cochons Island and Guillou Island. The Cochons Island sample included 38 mice trapped in 2009 (Program IPEV n°136, J.-L. Chapuis). Temporal variation in mandible shape has been documented in Guillou (Renaud et al. 2013), but does not notably affect the biomechanical parameters of the mandible (Renaud et al. 2015). Two time periods were therefore included in the present analysis, documenting the earliest and latest record available (1993, 18 mice and 2009, 22 mice) (Program IPEV n°136, J.-L. Chapuis). The sample from New Island from the Falklands (south-western Atlantic) included 15 mice captured in 2006 and 2010 by the team of Petra Quillfeldt. These Kerguelen and Falklands specimens were prepared and are currently stored at the LBBE, Lyon. The sample from Marion Island, off South Africa, was composed of 12 mice captured in 1997 (collection Institut des Sciences de l’Evolution, Montpellier, France). All of these islands are deprived of permanent human settlement and mice returned to a feral way of life, mainly relying on habitat driven food resources for their maintenance.

A large proportion of macroinvertebrate prey has been documented for mice from the Kerguelen (Le Roux et al. 2002) and Marion Island (Smith et al. 2002; van Aarde and Jackson 2007) based on stomach contents. Since a similar shift in diet has also been evidenced in the population from Macquarie Islands (Copson 1986), such foraging behavior was hypothesized for the Falkland population. For comparison, two commensal populations from Western Europe were considered: Gardouch, France and Cologne-Bonn, Germany (68 and 14 mice respectively; Gardouch: collection of the Centre de Biologie et Gestion des Populations, Montpellier, France; Cologne-Bonn: provided by the Max Plank Institute for Evolutionary Biology, Plön, Germany, prepared and currently stored at the LBBE, Lyon) (Renaud et al. 2015). All mice considered were sub-adults and adults, the criteria being the eruption of the third molars that occurs at weaning. Sexual dimorphism was not evidenced in mandible shape in such populations (Renaud et al. 2013). Hence, males and females were pooled for further analyses.

Mitochondrial D-loop sequences and 18 microsatellites corresponding to mice from these islands and Western European localities were retrieved from previously published studies (Ihle et al. 2006; Hardouin et al. 2010).

Methods

Phylogenetics

A phylogenetic tree was calculated using Mr. Bayes (Ronquist et al. 2012) and PhyML (Guindon et al. 2010) using the substitution model HKY+I+G infer using jmodeltest (Guindon and Gascuel 2003; Darriba et al. 2012). *M. m. musculus* (DQ266060) and *M. m. castaneus* (DQ266061) were used as outgroup. The generation number was set at 5 000 000 with 25% of burn-in. The tree was visualized using FigTree v1.3 (Rambaut 2012). The numbers of haplotypes and haplotype diversity per populations were calculated using DNAsp (Librado and Rozas 2009). Pairwise Fst values using the mitochondrial D-loop were calculated using Arlequin (Excoffier and Licher 2010).

The 18 microsatellites were analyzed using the package adegenet (Jombart 2008). The population structure was identified with a Discriminant Analysis of Principal Components (DAPC) (Jombart et al. 2010).

Mandible size and shape

Mandibles (Fig. 2A) were photographed using a Leica MZ stereomicroscope. The mandible shape was quantified by the 2D outline of the mandibular bone (Fig. 2B), the hemi-mandible being placed flat on its lingual side. The starting point of the outline was positioned at the upper connection between the incisor and the bone, and 64 points were sampled at equal curvilinear distance along the outline using the image analyzing software Optimas 6.5, from which 64 radii (distance from each point to the center of gravity) were calculated. This series was analyzed using a Fourier-based method, decomposing it into a sum of trigonometric functions of decreasing wavelength (harmonic), each weighted by two Fourier coefficients (FCs). The zero harmonic A0 was used as a size estimator and to standardize all other FCs. Seven harmonics (i.e. 14 FCs) were deemed sufficient for describing the mandible shape and filtering measurement error (Renaud and Michaux 2003).

By comparing function(s) of a curve, and not the points collected on the outline, Fourier methods allow investigation of shapes deprived of or with few landmarks with clear homology (Bonhomme et al. 2014; Dujardin et al. 2014). Regarding the mandible, most landmarks are located along the outline, and correspond to maxima of curvature (landmarks of type 2). An outline analysis captures this morphological information together with the curvature of the processes and anterior part. Compared to sliding semi-landmarks, outline analyses perform equally well (Sheet et al. 2006), but allow a reduction in the number of variables, by retaining first harmonics only. In the present case, it has the further advantage of quantifying mandible shape without relying on landmarks that were used for biomechanical estimates, thus avoiding any risk of redundancy between the two datasets.

Shape differences were described into the morphospace defined by the first axes of a principal component analysis (PCA) on the variance-covariance matrix of the FCs. Univariate differences between groups in mandible size were investigated using a Kruskal-Wallis test and associated pairwise Mann-Whitney tests using Past3 (Hammer et al. 2001).

The PCA was run using the package ade4 (Dray and Dufour 2007) in the R environment (R-Core-Team 2017). Multivariate differences in mandible shape between groups were tested using a permutational multivariate analysis of variance (Permanova; significance estimated based on 9999 permutations) on the 14 FCs using Past3 (Hammer et al. 2001).

Biomechanical analysis of the mandible

The mechanical advantage is a measure of the efficiency of mandible geometry to transmit force from the muscles to the bite point. It can be estimated as the ratio of the in-lever (distance from the condyle to the point of muscle attachment) and the out-lever (distance from the condyle to the bite point) (Hiiemae 1971). Out-levers (Fig. 2C) were estimated as the distance from the condylar articulation (playing here the role of fulcrum) to the incisor tip, and to the first molar hypoconid.

Three in-levers were measured (Fig. 2C). The effect of the deep masseter was approximated by the distance from the condyle to the anterior boundary of the angular process, towards the ventral margin of the masseteric fossa, where it attaches. The effect of the superficial masseter was approximated by considering the distance from the condyle to the posterior tip of the angular process. The distance from the condyle to the posterior tip of the coronoid described the action of the temporalis (Anderson et al. 2014; Renaud et al. 2015). The temporalis is mostly used together with incisors for gnawing, whereas the masseter and molars are involved in the action of mastication. The masseter also contributes to bringing the incisors into occlusion. Four mechanical advantages were therefore considered: temporalis/incisor, superficial master/ incisor, superficial masseter/molar, and deep masseter/molar.

In- and out-lever distances were calculated from landmark coordinates registered using TPSdig2 (Rohlf 2010a). This set of six landmarks was also investigated using geometric morphometrics. The coordinates were aligned, scaled and rotated using a generalized least squares Procrustes superimposition. A principal component analysis was performed on the resulting aligned coordinates using TPSrelw 1.49 (Rohlf 2010b). Visualization of shape changes between group means were obtained using the R package geomorph (Adams and Otarola-Castillo 2013).

Finally, the values of the in- and out-levers themselves were considered. To discard any effect of size differences between mandibles, they were computed from the aligned coordinates, hence being standardized by centroid size (e.g. the square root of the sum of squared distance of each landmark to the centroid of the configuration).

Univariate differences between groups regarding the in- and out-lever arms and the mechanical advantages were investigated using Kruskal-Wallis tests and associated pairwise Mann-Whitney tests using PAST3 (Hammer et al. 2001). The Kruskal-Wallis test is a non-parametric analogue of an analysis of variance. Being ultimately based on a ranking of the values, it is appropriate even for non-normal variables, such as ratios. Relationships between parameters were assessed using a Pearson's product-moment correlation estimated with R.

Measurement error

To assess how much importance of measurement error might impact the results, the mandible outline and the in- and out-lever distances of the 15 specimens from New Islands were measured twice, at an interval of several months. Differences between the two sets of measures were tested using Kruskal-Wallis tests for mandible size, in- and out-levers, and biomechanical ratios. The difference in mandible shape was tested using a permanova (9999 replications) on the 14 FCs and on the aligned coordinates of the biomechanical landmark configuration.

Influence of phylogeny and climate on morphology

The relative influences of phylogeny and climate on morphology were investigated using linear models. The variables to be explained were (1) mandible shape, described by the set of PC axes explaining more than 5% of variance, based on the outline analysis; and (2) the biomechanical ratios considered separately.

The explanatory sets of variables were constructed as follows:

1. Climatic data were extracted from the WorldClim database with a resolution of 2.5 arc-min using the raster package (Hijmans 2014). The 19 bioclimatic variables available were retrieved: Annual Mean Temperature, Mean Diurnal Range [Mean of monthly (max temp - min temp)], Isothermality, Temperature Seasonality (standard deviation *100), Max Temperature of Warmest Month, Min Temperature of Coldest Month, Temperature Annual Range, Mean Temperature of Wettest Quarter, Mean Temperature of Driest Quarter, Mean Temperature of Warmest Quarter, Mean Temperature of Coldest Quarter, Annual Precipitation, Precipitation of Wettest Month, Precipitation of Driest Month, Precipitation Seasonality (Coefficient of Variation), Precipitation of Wettest Quarter, Precipitation of Driest Quarter, Precipitation of Warmest Quarter, Precipitation of Coldest Quarter. These variables are based on average monthly climate data for minimum, mean, and maximum temperature and for precipitation for the period 1960-1990. They were summarized using a PCA on the correlation matrix. Axes explaining more than 5% of variance were kept in the model. These climatic data were used as a proxy of the local conditions, and hence, indirectly, of the food resources available to mice.

2. Phylogeny was first assessed using mitochondrial D-loop sequences. Fst distances were computed among the six groups (France, Germany, and the four Sub-Antarctic islands). A Principal Coordinate Analysis (PCOA) was performed on this distance matrix using ade4 (Dray and Dufour 2007). The set of axes > 5% were retained in the linear model.

(3) Phylogenetic relationships between islands and Western European localities were further assessed using the microsatellites data. The axes of the DAPC > 5% of variance were retained in the linear model. Because of possible redundancy between both phylogenetic data sets, separate models were built with D-loop and microsatellite data.

The percentage of variance explained (pve) by each set of explanatory variables and the associated p-value were assessed using the R package ffmanova (Langsrud and Mevik 2012). This method is based on type II sum of squares, which has the advantage of being invariant to ordering of the model terms; the ffmanova also handles colinear responses. It may inflate the pve but allows an estimation of the relative importance of the explanatory variables.

Results

Phylogeny

Regarding the D-loop data, the continental Western European groups were highly variable (Fig. 3A; Supp. Table 1), each including several of the main haplogroups described in the mouse (Bonhomme et al. 2011; Jones et al. 2013). In contrast, each island displayed a very restricted genetic diversity (Supp. Table 1), evidence of a founder effect, and subsequent resilience to late invaders in these remote environments (Hardouin et al. 2010). Each of the Sub-Antarctic populations considered has its own phylogeographic signature, underlining that each island was colonized independently from a different source population. The only exception is New Island (Falklands) and Guillou Island (Kerguelen), sharing a similar haplotype. These two populations differ, however, when considering their microsatellite signature (Hardouin et al. 2010) (Fig. 3B). Their common haplotypic signature may be the result of common source of colonization, related to the main harbors where whalers came from or made stop on their way to Sub-Antarctic oceans.

Measurement error

The two sets of measurements of the 15 New Island specimens did not differ in mandible size (A0: P = 0.852) nor shape (set of 14 FCs: P = 0.986). They did not differ for any of the scaled in- and out-lever measurements (Incisor: P = 0.548; Molar: P = 0.852; Coronoid: P = 0.548; tip of the angular process: P = 0.373; anterior boundary of the angular process: P = 0.191). As a consequence, none of the mechanical advantage differed between the two replicates (temporalis/incisor: P = 0.633; sup. masseter/ incisor: P = 0.351; sup. masseter/molar: P = 0.494; deep masseter/molar: P = 0.054).

Finally, the configuration of biomechanical landmarks did not differ between replicates (permanova on the aligned coordinates: P = 0.661).

Mandible size and shape

Mandible size was variable among mainland and insular populations (P < 0.0001; pairwise tests: Table 1). Mandibles were the largest on Marion Island. The smallest were documented on New Island (Falklands) and for the mice trapped on Guillou Island (Kerguelen) in the earliest record, 1993 (Fig. 4A). Western European populations were variable and overall intermediate between the insular ones.

Regarding mandible shape, three axes of the PCA on the Fourier coefficients explained more than 5% of the total variance (PC1: 49.8%, PC2 = 25.3; PC3: 11.8%, PC4 = 4.7%). On the first principal plane, populations from Western Europe were grouped on one side of the morphospace (Fig. 4B). All insular populations were different from this reference shape (permanova P < 0.0001 for all pairwise tests). Mandibles from the two Kerguelen islands, Cochons and Guillou, were shifted along the first axis (49.8% of total variance). Mandibles from Guillou caught in 1993 were the most divergent along this axis. Mandibles from New Island (Falklands) were divergent mostly along the second axis (25.3%). Marion Island was slightly divergent from Western Europe along the second axis but mostly along the third axis (11.8%) together with Cochons Island (Kerguelen) (data not shown).

These differences, although statistically highly significant, were subtle in terms of shape (Fig. 4C). Mandibles from Guillou, Cochons and New Island tended to display a reduced angular process. Guillou mandibles also had a ventrally narrower molar zone than continental mice. Marion mandibles displayed a pronounced angular process, originating from a smooth ventral edge of a ventrally narrow molar zone.

Biomechanics

First, the geometric morphometric analysis of the six biomechanically relevant landmarks (Fig. 5) provided an image of the differentiation between populations close to the one delivered by the outline analysis. Continental mandibles cluster together, and Sub-Antarctic populations differ in different directions around this cluster. Similar to the outline analysis, Marion and Guillou mandibles are the most differentiated, New Island mandibles being rather intermediate. Based on the six landmarks, Cochons mandibles fall close to New Island ones. These two populations share a backward shift of the tip of the angular process together with an anterior shift of its anterior edge. Marion mandibles share a posteriorly shifted tip of the angular process, but associated with a forward shift of the coronoid tip. Guillou mandibles display an anterior shift of the angular anterior edge, but associated with a backward shift of the coronoid tip.

These geometric differences translated into differences in the scaled in- and out-lever arms (Fig. 6; Table 2). The most consistent patterns shared by all Sub-Antarctic populations and differentiating them from continental ones were: (1) an increased incisor out-lever. A longer out-lever arm favors speed to the detriment of force at the point of occlusion. (2) An increased temporalis in-lever arm. (3) A decreased in-lever arm characterizing the tip of the angular process, approximating the action of the superficial masseter.

These differences in out- and in-lever arms had consequences on the mechanical advantages (MA) characterizing the main systems for biting (Fig. 7; Table 3). The most consistent pattern is displayed by the superficial masseter/incisor MA, for which all islands were highly significantly below continental values, but did not differ between them (Table 3). All islands also strongly differed from the continental values for the superficial masseter/molar MA, islands displaying lower values than the continent, but differences existed between islands. The temporal/incisor MA tended to be higher in Sub-Antarctic mice than on the continent, but this difference was less pronounced for Marion island. Finally, the deep masseter/molar MA was the less consistent among islands, with Cochons and New Island displaying values similar to the continent.

Overall, this resulted in a negative relationship between the temporal/incisor and superficial masseter/molar MA (Fig. 7E) ($R = -0.584$, $P < 0.001$). This relationship may reflect a trade-off existing even within populations, since a similar relationship was evidenced within the well-sampled population from Gardouch ($R = -0.318$, $P = 0.008$).

Relationship between morphology, phylogeny and climate

Models considering mandible geometry and biomechanical properties in relation to phylogeny and climate were investigated. Sets of variables to be explained were defined as follow. (1) Mandible shape was described by the first three axes of the PCA on the 14 FCs, these axes explaining more than 5% of variance (see above). (2) Several biomechanical advantages were further considered separately in relation to phylogeny and climate. Regarding explanatory variables, the sets of variables were designed as follow. (1) Environmental conditions were summarized by the first three axes of a

PCA on the 19 bioclimatic variables of WorldClim. These three axes explained more than 5% of variance (63.0%, 25.9%, 8.5%). The climate clearly opposes the continental localities to all Sub-Antarctic islands, Marion displaying the most extreme conditions. This set of variables will thus tend to characterize the Sub-Antarctic environment vs. continental conditions. (2) The phylogeny based on D-loop sequences was summarized by the first three axes of a PCOA on the Fst matrix (Supp. Table 2), all explaining more than 5% of variance (48.7%, 32.2%, 18.9%). (3) The phylogenetic relationships based on the microsatellites were summarized by the first three axes of the DAPC on the 18 microsatellites (68.2%, 21.0%, 6.4% of variance, respectively).

Considering first phylogeny estimated by the D-loop, the model for mandible shape indicated a primary influence of phylogeny (12.6%) and a lesser influence of climate (9.4%), both factors being significant.

Regarding the mechanical advantages, all were primarily correlated with climate and only secondarily with phylogeny (temporalis/incisor: climate = 18.1%, phylogeny = 9.6%; deep masseter/molar: climate = 22.3%, phylogeny = 14.0%; superficial masseter/molar: climate = 37.5%, phylogeny = 10.4%; superficial masseter/incisor: climate = 17.9%, phylogeny = 3.1%).

These results were corroborated when considering the phylogenetic relationships based on microsatellites. Phylogeny explained 12.6% of mandible shape, whereas climate explained only 6.1%. Mechanical advantages were all better explained by climate (temporal/incisor: climate = 18.1%, microsatellites = 9.6%; deep masseter/molar: climate = 17.4%, microsatellites = 14.0%; superficial masseter/molar: climate = 28.5%, microsatellites = 10.4%; superficial masseter/incisor: climate = 13.1%, phylogeny = 3.1%).

Discussion

Divergence in mandible shape primarily influenced by phylogeny

This study demonstrates a divergence of mouse jaws in these four Sub-Antarctic islands when compared to the Western European continental morphology. This matches previous results showing a divergence of insular jaw morphologies in settings as diverse as Faroe in the North Atlantic (Davis 1983), and Corsica and Sardinia in the Mediterranean Sea (Renaud and Auffray 2010). The phylogenetic source of the founding population appeared of primary importance in the diversification. These results echo recent findings on mouse tooth shape (Ledevin et al. 2016), suggesting that constraints related to the set of founder individuals constrain the subsequent diversification. As a consequence, despite a significant role of environmental conditions driving divergence, mandible shape from the different Sub-Antarctic populations did not diverge from the continental Western Europe towards a common morphology. Each population displayed its own idiosyncratic morphological signature.

Surprisingly, mandible size did not display a coherent increase in insular populations. Some populations (New Island from [Falklands], Guillou [Kerguelen]) even displayed smaller mandible size than continental populations. Covariation between mandible and body size has been repeatedly evidenced, between and within populations, in rodents (Cardini and Tongiorgi 2003; Renaud 2005) including house mice (Renaud et al. 2017). If mandible size is considered as a rough estimate for body size, it might have been expected to increase due to the combined effect of two well-known rules. First, the Bergman's rule predicts increased body size in mammals towards high latitude (Meiri and Dayan 2003). Second, the insular rule predicts that small mammals should become larger on islands (Lomolino 1985, 2005). However, the results suggest no consistent trends in size despite the supposed combination of the Bergman's and island rules regarding our insular samples. Possibly, the mice are close to their physiological limits in Sub-Antarctic environments (Berry et al. 1978), and low availability in resources of quality may limit growth in body and even investment in skeletal traits such as the mandible (Renaud et al. 2015). Such limitation may vary from island to island, explaining the range of variation from the small Guillou and the large Marion mandibles.

Functional adaptation to an increased role of prey catching

To focus on potential adaptive traits, the mandible geometry was also described by a simple set of landmarks describing functionally relevant in- and out-levers. The geometry of this landmark configuration shows, as the outline analysis, continental mice from Western Europe sharing a similar zone of the morphospace, and Sub-Antarctic populations diverging from them in different directions. Guillou and Marion mandibles appear, once again, the most different among Sub-Antarctic ones.

However, when considering in- and out-lever arms based on this geometry, some consistent patterns emerged. Sub-Antarctic mice share an increase in the incisor out-lever. Such increased out-lever arm is unfavorable to bite force, but favors velocity. Such trait facilitates the capture of prey and accordingly, an elongated rostrum has been evidenced in insectivorous rodents (Samuels 2009). Sub-Antarctic mice further share an increase in the temporalis in-lever arm. The temporalis plays a role in moving incisors into occlusion (Baverstock et al. 2013), another important aspect for catching prey. An increase in the in-lever arm is favorable to increased bite force, and may compensate the elongation of the out-lever. Sub-Antarctic mandibles also tend to share a decrease in the superficial masseter in-lever, although this decrease is less pronounced for Marion mandibles. The molar out-lever and the deep masseter in-lever do not show consistent trends among Sub-Antarctic mice, evidencing their mosaic divergence from the continental stock.

As a consequence of these differences in the in- and out-lever values, consistent differences characterizing Sub-Antarctic mice also emerged when considering their ratios, i.e. mechanical advantages, characterizing the biomechanical efficiency of the mandible tool. Three consistent trends were evidenced. First, despite the increase of the incisor out-lever, the temporalis/incisor MA is increased in Sub-Antarctic mice. In contrast, the superficial masseter/incisor MA is decreased. Increased action of the temporalis and decreased contribution of the masseter have been described in carnivorous murine rodents (Fabre et al. 2017). They may contribute, together with the increased incisor out-lever arm, to an action favoring speed instead of force at incisor biting. This constitutes an adaptation to the food resources of Sub-Antarctic mice, which largely prey on macro-invertebrates (Copson 1986; Le Roux et al. 2002; Smith et al. 2002; van Aarde and Jackson 2007). Sub-Antarctic mice also share a decrease in the superficial masseter/molar MA, which seems detrimental to exert force at molar biting. Possibly, this aspect related to chewing resistant food became less important than in continental mice, which being commensal, mostly rely on seeds and other items of vegetal origin in an agricultural context.

The different Sub-Antarctic populations do not share exactly the same resources, being context dependent on the availability of local fauna and flora, plus is influenced by climate, for instance preventing access to subterraneous invertebrates such as earthworms (Le Roux et al. 2002). Some Sub-Antarctic populations even include vertebrate prey, such as chicks of sea birds, in their diet (Cuthbert and Hilton 2004). These differences may contribute to explain why the different populations did not achieve exactly the same biomechanical signature. Adaptation to local food resources may further include the muscle architecture (Satoh and Iwaku 2006), or even the digestive system (Samuels 2009), but the role of these aspects in adaptation at the intra-specific level remain to be documented.

Adaptive mechanical convergence despite morphological differentiation

The mandibles of the different Sub-Antarctic islands thus display a similar adaptive shift in functional aspects that does not echo any similar evolution in shape (Alfaro et al. 2004; Wainwright 2007). Shall this evolutionary pattern be termed convergence, or parallelism? Considering that continental mice share a similar morphology, the repeated evolution from this common ancestor morphology of 'insectivorous-like' biomechanical properties may be termed parallelism. However, continental populations also displayed some differences, and the phylogenetic data clearly show that the ancestral stocks invading each island were indeed different. We therefore favor the term of convergent evolution, although in the present case, parallelism and convergence may be very close.

Tools can achieve the same function even when having differences in shape, leading to a many-to-one mapping of form to function (Wainwright et al. 2005; Wainwright 2007; Losos 2011). The mouse mandible may display here such a complex relationship between shape and function, exemplifying results of modelling suggesting that convergence can be demonstrated only when considering a simplified genotype-phenotype map (Salazar-Ciudad and Marín-Riera 2013). Morphological details, such as those captured by the morphometric analysis, may trace phylogenetic idiosyncrasy that are not of functional relevance and thus not prone to adaptive evolution, corresponding to 'neutral morphological evolution' (Wainwright 2007). They may also correspond to different ways to achieve the same functional change, and/or correspond to different fine tuning to local resources. In contrast, considering simple ratios, such as the mechanical advantage, may place the focus on functionally relevant features prone to the detection of adaptive convergence.

Indeed, many documented instances of convergent evolution rely on simple morphological estimates, such as jaw length in snakes (Aubret et al. 2004), in- and out-levers in cichlid fishes' jaws (Albertson et al. 2003), number of plates in sticklebacks (Marchinko and Schluter 2007) or limb length in lizards (Losos et al. 1997; Calsbeek and Irschick 2007). When complex traits are considered, multidimensional aspects of the niche partitioning may be involved (Harmon et al. 2005), further complicating the identification of convergent evolution.

Decrease of performance of other functions of the mandible

While our results indicate an adaptive improvement in the functioning of biting at the incisors, we observed at the same time a decrease in the mechanical advantage associated with chewing at the molars (Baverstock et al. 2013). This masseter/molar complex is used primarily in the consumption of hard or resistant food items. This type of functioning should be important for commensal mice feeding mostly on grains in agricultural buildings, but should become less relevant for Sub-Antarctic mice relying on other food resources. The decrease in the masseter / molar mechanical advantage is thus probably related to a relaxation of the pressure on this function. Previously observed on Guillou Island (Kerguelen) (Renaud et al. 2015), this decrease in performance of the masseter / molar complex appears as a general feature of the Sub-Antarctic mice. Beyond the relaxation of the pressure related to mastication, this decrease in performance may correspond to a trade-off between incisor and molar biting. Since the negative relationship between the temporalis/incisor and masseter/molar mechanical advantages is also displayed at the intra-population level, it supports the idea that optimizing one of the functions is detrimental to the other. The mandible of omnivorous murine rodents is known as a paradigm example of a versatile generalist tool adapted to all feeding modes (Cox et al. 2012). Yet, species specializing towards carnivory or insectivory display specific adaptations that modulate this generalist morphology (Samuels 2009; Fabre et al. 2017). The case of the Sub-Antarctic mice suggests that such fine-tuning may occur even at the intra-specific level.

Plasticity and/or heritable changes?

The question arises of the mechanisms involved in this convergent biomechanical response. Only experiments could definitely answer this issue, but these are difficult for animals from such remote places. A comparison with a former experiment on laboratory mice may however shed some light on this aspect. Inbred laboratory mice were bred from weaning up to six months on food of different consistency: one group was fed the regular rodent pellets (considered as hard food), another group the same food served as jelly (soft food). This difference in food consistency triggered a change in mandible shape (Renaud and Auffray 2010). This shape change was shown to have mechanical consequences: the temporalis/incisor and masseter/molar mechanical advantages both decreased in the mice served food as jelly (Anderson et al. 2014). For both mechanical advantages, the decrease was by 3-6% (Anderson et al. 2014). This was interpreted as a difference in bone remodeling that occurs in response to stimulations by muscle activity. Mandibles subjected to less activity resulted in less efficient morphologies.

The decrease in performance observed for the masseter/molar complex in Sub-Antarctic mice (-3.2% for Marion Island up to -5.7% for Guillou Island [Kerguelen] in 2009) falls within the range of values compatible with the plastic response observed in laboratory mice. In contrast, the increase in performance for the temporalis/incisor complex (+7.2% for Marion Island up to +20.1% for New

Island [Falklands]) by far exceeds the plastic change in laboratory mice, which was however related to a substantial change in food consistency (Anderson et al. 2014).

This adaptive increase in performance of the temporalis/incisor complex may not be only due to plasticity. The occurrence of the convergent response in several independent cases indicates that directional selection related to a similar diet shift likely drove this morphological change. Even if plastic response allows the first step of response following invasion, genetic assimilation will likely take over and lead to selection for gene coding for the new morphology (Aubret and Shine 2009). Sub-Antarctic islands have been colonized by explorers and whalers in the course of the 19th century [e.g. (Frenot et al. 2001)], and evidence of mice on these islands date back to the middle of the 19th century (Kidder 1876). Thus, mice had more than hundred years to evolve, and thus probably more than 500 generations (considering a generation time of three or four generations per year). This time lapse is short compared to usual evolutionary scale, but large compared to recent findings of contemporary evolution (Reznick and Ghalambor 2001; Collyer et al. 2007; Kinnison and Hairston 2007).

In contrast, the masseter/molar mechanical advantage decreased by about what would be expected for a plastic response, based on the laboratory experiment (Anderson et al. 2014). Since no directional selection was exerted on it, and instead there was a release of selection, the morphological signal may simply correspond to a plastic response, due to less muscular stress exerted on the mandible in relation with a decrease of the consumption of hard / resistant food.

Conclusions

The results indicate a convergent adaptive evolution of the biomechanical function of the jaw of Sub-Antarctic mice, related to their shift towards a diet enriched with invertebrate prey. Yet, the adaptive component of this morphological change was only evidenced when considering simple but mechanically relevant in- and out-levers, and their ratios. When considering shape in all its complexity, the dominant signal was the idiosyncrasy of each insular population, related to its history of colonization and possibly, fine-tuned response to local resources. The amount of adaptive morphological change appears larger than expected for a plastic response due to bone remodeling under the action of the masticatory muscles. This suggests that even if plasticity contributed in the first step of the mouse establishment on a new island, genetic assimilation likely took place over the century or more of insular evolution. This complex relationship between shape evolution and the adaptive response may render the identification of underlying genetic changes more complex than for more simple traits. Possibly, each island reached adaptive morphology by the selection of different genes and by tinkering the gene pool inherited from the founder population.

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Tables

	Mean A0	σ(A0)	Continent	Guillou 1993	Guillou 2009	Cochons	New Island	Marion
Continent	27.5	3.5	-					
Guillou 1993	28.8	1.6	**< 0.001**	-				
Guillou 2009	29.4	2.0	0.056	*0.017*	-			
Cochons	28.5	2.2	*0.016*	**< 0.001**	**0.002**	-		
New Island	30.1	2.0	0.072	0.086	0.938	**0.002**		
Marion	32.4	2.0	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	-

Table 1. Size differences between mandibles of the different populations. Size is estimated by A0 provided by the Fourier analysis. Group mean and standard deviation (σ) are provided in the first two columns. Probabilities of two-by-two Mann-Whitney tests are provided (in bold P ≤ 0.01; in italics P ≤ 0.05).

		Mean	σ	Continent	Guillou 1993	Guillou 2009	Cochons	New Island
Out-Inc	Continent	1.072	0.011	-				
	Guillou 1993	1.097	0.008	**< 0.001**	-			
	Guillou 2009	1.088	0.007	**< 0.001**	**0.001**	-		
	Cochons	1.104	0.009	**< 0.001**	*0.021*	**< 0.001**	-	
	New Island	1.101	0.008	**< 0.001**	0.277	**< 0.001**	0.435	-
	Marion	1.098	0.010	**< 0.001**	0.871	**0.007**	0.086	0.421
Out-Mol	Continent	0.549	0.017					
	Guillou 1993	0.551	0.010	0.950				
	Guillou 2009	0.544	0.007	0.063	*0.024*			
	Cochons	0.567	0.009	**< 0.001**	**< 0.001**	**< 0.001**		
	New Island	0.568	0.009	**< 0.001**	**< 0.001**	**< 0.001**	0.775	
	Marion	0.562	0.016	*0.034*	*0.027*	**< 0.001**	0.199	0.180
In-Temp	Continent	0.198	0.018					
	Guillou 1993	0.223	0.024	**< 0.001**				
	Guillou 2009	0.223	0.014	**< 0.001**	0.802			
	Cochons	0.239	0.015	**< 0.001**	*0.020*	**0.001**		
	New Island	0.246	0.018	**< 0.001**	**0.008**	**< 0.001**	0.273	
	Marion	0.217	0.024	**0.004**	0.444	0.439	**0.005**	**0.002**
In-SMass	Continent	0.357	0.014					
	Guillou 1993	0.337	0.011	**< 0.001**				
	Guillou 2009	0.331	0.009	**< 0.001**	0.114			
	Cochons	0.337	0.010	**< 0.001**	0.643	**0.007**		
	New Island	0.333	0.011	**< 0.001**	0.514	0.259	0.158	
	Marion	0.343	0.017	**0.009**	0.186	*0.012*	0.137	0.092
In-DMass	Continent	0.486	0.013					
	Guillou 1993	0.474	0.012	**0.002**				
	Guillou 2009	0.460	0.009	**< 0.001**	**0.001**			
	Cochons	0.500	0.010	**< 0.001**	**< 0.001**	**< 0.001**		
	New Island	0.495	0.012	*0.038*	**< 0.001**	**< 0.001**	0.158	
	Marion	0.502	0.018	**< 0.001**	**0.001**	**< 0.001**	0.207	0.124

Table 2. Scaled in- and out-lever values, calculated on the aligned coordinates after Procrustes superimposition, of the mandibles of the different populations, and differences between groups. Inc = incisor; Mol = molar; Temp = temporalis; SMass = superficial masseter; DMass = deep masseter. P-values of two-by-two Mann-Whitney tests are provided (in bold P ≤ 0.01; in italics P ≤ 0.05). Group mean and standard deviation (σ) are provided in the first two columns.

		Mean MA	σ(MA)	Continent	Guillou 1993	Guillou 2009	Cochons	New Island
MA Temp/Inc	Continent	0.185	0.016	-				
	Guillou 1993	0.203	0.020	**0.001**	-			
	Guillou 2009	0.205	0.012	**< 0.001**	0.918	-		
	Cochons	0.216	0.013	**< 0.001**	*0.027*	*0.005*	-	
	New Island	0.223	0.015	**< 0.001**	**0.005**	**< 0.001**	0.164	-
	Marion	0.198	0.021	*0.014*	0.444	0.340	**0.008**	**0.001**
MA SMass/Inc	Continent	0.333	0.015	-				
	Guillou 1993	0.307	0.009	**< 0.001**	-			
	Guillou 2009	0.304	0.009	**< 0.001**	0.308	-		
	Cochons	0.305	0.010	**< 0.001**	0.563	0.524	-	
	New Island	0.303	0.011	**< 0.001**	0.149	0.676	0.309	-
	Marion	0.312	0.018	**< 0.001**	0.275	0.069	0.120	0.102
MA SMass/Mol	Continent	0.651	0.037	-				
	Guillou 1993	0.612	0.019	**< 0.001**	-			
	Guillou 2009	0.609	0.019	**< 0.001**	0.545	-		
	Cochons	0.595	0.019	**< 0.001**	**0.004**	**0.008**	-	
	New Island	0.587	0.022	**< 0.001**	**0.004**	**0.006**	0.203	-
	Marion	0.610	0.035	**0.001**	0.659	0.928	0.184	0.092
MA DMass/Mol	Continent	0.885	0.027	-				
	Guillou 1993	0.861	0.021	**0.001**	-			
	Guillou 2009	0.846	0.015	**< 0.001**	*0.028*	-		
	Cochons	0.883	0.016	0.486	**0.001**	**< 0.001**	-	
	New Island	0.871	0.016	*0.037*	0.138	**< 0.001**	0.062	-
	Marion	0.893	0.028	0.269	**0.005**	**< 0.001**	0.143	*0.016*

Table 3. Biomechanical ratios characterizing the mandibles of the different populations, and differences between groups. MA Temp/Inc = Mechanical advantage Temporalis / Incisor; MA SMass/Inc = Mechanical advantage Superficial Masseter / Incisor; MA DMass/Mol = Mechanical advantage Deep Masseter / Molar. P-values of two-by-two Mann-Whitney tests are provided (in bold P ≤ 0.01; in italics P ≤ 0.05). Group mean and standard deviation (σ) are provided in the first two columns.

Figure captions

Figure 1. Map of the localities considered in this study.

Figure 2. (A) Examples of mandibles of the house mouse (*Mus musculus domesticus*) in Western Europe and the different Sub-Antarctic islands. B. Outline of the mandible, used for the Fourier analysis providing the shape variables. The dot represents the starting point. C. Biomechanical variables used to quantify the functional performance of the mandible. The condyle being the fulcrum, in-lever corresponded to the distance from this fulcrum to the zone of muscle insertions; out-lever corresponded to the distance from the fulcrum to the bite point.

Figure 3. (A) Bayesian phylogenetic tree based on D-loop sequences. The posterior probabilities as well as bootstrap values are displayed on the branches. The sequences included roughly correspond to the morphometric sampling areas. (B) Representation of the microsatellite variation on the first three axes of a DAPC.

Figure 4. Morphological variation of the mandible in the Sub-Antarctic islands and two commensal populations. (A) Mandible size. Each dot corresponds to a specimen. (B) Mandible shape differentiation in the morphospace based on the outline analysis of the mandible. Ellipses correspond to the 95% confidence interval around the centroid. Populations: Western Europe (CB: Cologne-Bonn; GAR: Gardouch); Sub-Antarctic islands: New Island, Falklands (NI); Cochons (COCH) and Guillou (G93: 1993 and G09: 2009) in the Kerguelen archipelago; Marion Island (MAR).

Figure 5. Geometric variations of the biomechanical landmark configuration between the Sub-Antarctic islands and two commensal populations. Middle panel, geometric differentiation in the morphospace based on the six biomechanically relevant landmarks. Ellipses correspond to the 95% confidence interval around the centroid. Populations: Western Europe (CB: Cologne-Bonn; GAR: Gardouch); Sub-Antarctic islands: New Island, Falklands (NI); Cochons (COCH) and Guillou (G93: 1993 and G09: 2009) in the Kerguelen archipelago; Marion Island (MAR). The other panels represent the deformation from the continental consensus configuration to the consensus geometry of each island (deformation magnified x2).

Figure 6. In- and out-lever arms describing the main biomechanical properties of the mandible geometry. Out-levers were estimated as the distance from the condylar articulation to (1) the incisor tip, and to (2) the first molar main cusp (hypoconid). In-levers were the distances from the condyle to: (1) the tip of the coronoid (describing the action of the temporalis); (2) the tip of the angular process (approximating the action of the superficial masseter); (3) the anterior boundary of the angular process (approximating the deep masseter action).

Figure 7. Biomechanical variation of the mandible in Sub-Antarctic and two commensal continental populations. Mechanical advantages (= In/Out lever arms) are: (A) temporalis/incisor; (B) superficial masseter / molar; (C) superficial masseter / molar; (D) superficial masseter / incisor. (E) Relationship between two mechanical advantages: superficial masseter/molar vs. temporalis/incisor.