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Impact of hybridization on shape, variation and covariation of the mouse molar --Manuscript Draft--

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Abstract:	<p>Hybridization can generate phenotypes departing from the parental range through many processes: increased variance due to increased heterozygosity, decreased phenotypic integration and altered covariation structure due to changes in the expression of (co)variation generating developmental processes, and transgression that may arise from epistasis and compensatory genes. Morphometric assessment of shape differentiation, variance and covariance may shed light on these processes, especially for complex characters for which the genetic basis is still far to be assessed. The shape of the first upper molar was thus quantified in a cross between inbred strains of the two European subspecies of the house mouse (<i>Mus musculus domesticus</i> and <i>M. m. musculus</i>). Hybrids appeared as moderately transgressive. Morphological variance was increased in F2s, back to levels observed in wild populations. The pattern of variance-covariance was different between the two parental strains, but restored to a wild-type structure in F2s. Finally, F2s displayed a morphological integration comparable to wild populations but lower than observed in the parental strains. This is interpreted as the result of the reshuffling of the standing genetic variation in hybrids that should have restored the expression of (co)variance generating processes made ineffective in parents due to random allele fixation of some loci. Inter-parental differentiation was more important in regions of the tooth developing early during embryogenesis, whereas transgression was more pronounced in late developing regions. Mutations may more easily generate important geometric differences early during tooth development, occurring as a cascade of signalization from the first cusp to initiate onward. Epistasis and constraints of neighboring parts may be more important later, promoting transgression and impeding differentiation.</p>

1 **Impact of hybridization on shape, variation and covariation of the mouse molar**

2

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4

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11

12 **Abstract**

13 Hybridization can generate phenotypes departing from the parental range through many processes:
14 increased variance due to increased heterozygosity, decreased phenotypic integration and altered
15 covariation structure due to changes in the expression of (co)variation generating developmental
16 processes, and transgression that may arise from epistasis and compensatory genes. Morphometric
17 assessment of shape differentiation, variance and covariance may shed light on these processes,
18 especially for complex characters for which the genetic basis is still far to be assessed.

19 The shape of the first upper molar was thus quantified in a cross between inbred strains of the two
20 European subspecies of the house mouse (*Mus musculus domesticus* and *M. m. musculus*). Hybrids
21 appeared as moderately transgressive. Morphological variance was increased in F2s, back to levels
22 observed in wild populations. The pattern of variance-covariance was different between the two
23 parental strains, but restored to a wild-type structure in F2s. Finally, F2s displayed a morphological
24 integration comparable to wild populations but lower than observed in the parental strains. This is
25 interpreted as the result of the reshuffling of the standing genetic variation in hybrids that should
26 have restored the expression of (co)variance generating processes made ineffective in parents due to
27 random allele fixation of some loci. Inter-parental differentiation was more important in regions of
28 the tooth developing early during embryogenesis, whereas transgression was more pronounced in
29 late developing regions. Mutations may more easily generate important geometric differences early
30 during tooth development, occurring as a cascade of signalization from the first cusp to initiate
31 onward. Epistasis and constraints of neighboring parts may be more important later, promoting
32 transgression and impeding differentiation.

33

34 **Keywords**

35 *Mus musculus domesticus* – house mouse – geometric morphometrics – transgressive phenotypes –
36 morphological integration

37

38

39

40 Introduction

41 Hybridization has often been considered as a process eroding diversity by diluting specific
42 differences. Exemplifying this aspect of hybridization, several native species are endangered by
43 hybridization with phylogenetically closely related species (Oliveira et al. 2008; Randi 2008).
44 Hybridization, however, has also the potential to generate novel phenotypes, up to contributing to
45 speciation and radiation processes (Amaral et al. 2014; Bell and Travis 2005; Dittrich-Reed and
46 Fitzpatrick 2013; Larsen et al. 2010). Several aspects are involved. (1) Genetic variance can simply be
47 increased by recombining parental genomes. This should lead to an increased phenotypic variance in
48 hybrids that may fuel the screening by selection. This does not necessarily mean, however, that the
49 mean morphology of hybrids would depart of being intermediate between parental lines. (2)
50 Hybridization may produce variation qualitatively different from that of the parental population. This
51 would be the case if the covariance structure is altered, releasing integration and promoting
52 evolvability (Parsons et al. 2011; Selz et al. 2013). (3) Hybridization can produce extreme phenotypes,
53 or transgressive phenotypes, compared to phenotypes observed in the parental lines (Albertson et
54 al. 2005; Nolte and Sheets 2005). The mean morphology of the hybrids is then shifted away from the
55 'expected' mid-shape between parents. Transgressive phenotypes can be produced in various ways.
56 Alleles with antagonist effects accumulated in parents (Rieseberg et al. 1999) can produce
57 combinations outranging those displayed in the parents once recombination occurred in F2s. The
58 combination of traits responding differently to hybridization, for instance mixing dominance for one
59 parent on a trait, dominance for the other on another trait, and additive response in a third trait, will
60 lead to original combination as soon as the F1 generation (Renaud et al. 2012). Finally, epistasis can
61 also promote transgression (Stelkens and Seehausen 2009).

62 Deciphering the impact of hybridization at a genetic and developmental level is still beyond of reach
63 for complex, highly multigenic characters. However, assessing the morphological signature of
64 hybridization on such characters may help to understand which, among the aforementioned
65 processes, contribute to the production of novel phenotypes in a set of hybrids: increased variance,
66 altered covariance structure, and/or transgression. Especially, integration refers to the tendency to
67 generate covariation between traits through developmental processes, but its expression is
68 intimately related to the presence of variation that makes it expressed and detectable (Hallgrímsson
69 et al. 2009). Because hybridization changes levels and sources of variation, hybrids provide a
70 particularly well suited model to increase our understanding about integration processes.

71 The hybrid zone between the two European subspecies of the house mouse, *Mus musculus*
72 *domesticus* and *M. m. musculus*, is an emblematic case of hybridization (Boursot et al. 1993; Sage et
73 al. 1993; Raufaste et al. 2005). Mandible and molar teeth of the house mouse have been the focus of

74 extensive evo-devo studies, providing a good background for understanding the basis of the
75 phenotypic variation. The mandible is known to be composed of well-identified developmental and
76 functional modules (Klingenberg et al. 2003; Klingenberg and Navarro 2012). Hybrid mice have been
77 shown to display transgressive mandible morphologies, largely due to a modular response to
78 hybridization generating original combinations in the hybrids (Renaud et al. 2012). This was
79 associated, though, to a moderate increase in phenotypic variance along a conserved direction of
80 (co)variance, at least in the F1s (Renaud et al. 2009).

81 In contrast, the molar tooth should not be modular. It develops as a cascade of signaling interactions
82 from the first cusp to initiate onward (Jernvall and Thesleff 2000). No modular response to
83 hybridization is thus expected. However, the genotype-phenotype map is probably very complex
84 along this spatio-temporal cascade. Genetic differences between parents and determinants of
85 variation may not have the same phenotypic outcome when occurring early or late during tooth
86 development.

87 We thus investigated the consequences of hybridization between the two European subspecies of
88 the house mouse on the morphology of the first upper molar (UM1). Molar shape, variance and
89 covariance structure were measured in a cross between wild-derived laboratory strains, including the
90 two parental groups, F1 and F2 hybrids, and backcrosses. Molar morphology was quantified using a
91 2D outline analysis based on points regularly spaced along the outline. Using this sampling, we
92 further developed a regional approach of shape, decomposing the tooth into regions being patterned
93 at different stages of the developmental cascade. The impact of hybridization could thus be
94 compared along the cascade. Finally, variance and covariance of these laboratory-bred mice were
95 compared to wild populations of the Western European house mouse *M. m. domesticus* to assess
96 how inbreeding may affect the outcome of hybridization in our laboratory model.

97

98 **Material and Methods**

99 **Material**

100 **Laboratory hybrid mice**

101 The mice from the two parental groups belonged to strains bred from wild-trapped animals and
102 conserved at the Wild Mouse Genetic Repository of Montpellier (Institut des Sciences de l'Evolution,
103 France). The Western European house mouse *M. musculus domesticus* was represented by 33 mice
104 from the strain WLA, derived from mice caught near Toulouse (France) in 1976. The Eastern

105 European subspecies *M. musculus musculus* was represented by 27 mice from the strain PWK,
106 derived from mice trapped in Prague (Czech Republic) in 1982.

107 38 F1 hybrids were bred from these parental groups, from both the WLA × PWK and the PWK × WLA
108 pairs. In order to buffer among-pair differences and to increase sample size, all F1 were considered
109 together. A total of 80 F2 hybrids, 81 backcrosses to WLA (BC-WLA) and 82 backcrosses to PWK (BC-
110 PWK) were further bred from these F1 hybrids (Fig. 1).

111 All 341 animals were bred in the same controlled conditions at the ISEM (Montpellier, France;
112 authorization certificate 04143 (03/26/1991) to JCA of the Ministère de l'Agriculture et de la Forêt
113 [French Ministry of Agriculture and Forest]). Both the PWK and WLA strains have been established
114 and maintained by brother/sister mating at the Institut Pasteur (Paris, France) to obtain inbred wild-
115 derived mouse strains and had been maintained in these breeding conditions for more than 50
116 generations at the time of the experiment.

117 **Wild-trapped mice**

118 Three well-sampled populations of wild-trapped mice (Renaud et al. 2015) were considered as
119 comparison to the laboratory strains. These populations are: (1) Gardouch, Southern France (N = 68);
120 (2) Fango Valley, Corsica, Mediterranean Sea (N = 53); (3) Marion Island, Sub-Antarctic Indian Ocean
121 (N = 92). The three populations documented to the Western house mouse *M. musculus domesticus*.
122 They are housed in the collections of the Institut des Sciences de l'Evolution, Montpellier, France (for
123 Fango and Marion) and the Centre de Biologie et Gestion des Populations, Baillarguet, France (for
124 Gardouch).

125 All individuals were considered as adults and sub-adults based on the criterion of the full eruption of
126 the third molars, occurring at weaning. Males and females were pooled since no sexual dimorphism
127 has been documented for tooth morphology in house mice (Renaud et al. 2013; Valenzuela-Lamas et
128 al. 2011).

129 **Methods**

130 **Outline analysis**

131 The first upper molar shape was approximated by the 2D outline of the tooth seen from the occlusal
132 surface, the focus being made towards the base of the crown, which is only affected by heavy wear
133 (Renaud 2005). Each outline was defined by a set of 64 points, the starting point being tentatively
134 positioned at the anteriormost part of the tooth. This set of points was first analyzed using an Elliptic
135 Fourier transform (Kuhl and Giardina 1982). This method describes the outline as its x- and y-
136 variations, as a function of the cumulative length along the outline, decomposed into a sum of

137 trigonometric functions of decreasing wavelength (harmonics) by a Fourier approach. Each harmonic
138 is weighted by four Fourier coefficients (FCs), two for x- and two for y-variations. This method allows
139 the alignment of the outline along the first axis of the ellipse best fitting the outline, and the
140 adjustment of the starting point at the intersection of the outline with this first axis, corresponding to
141 the major elongation of the object. A drawback is a high number of variables required to describe the
142 outline (four FCs by harmonics). This method was used to reconstruct an outline with the starting
143 point adjusted along the first axis of the major ellipse. This outline was then analyzed using a Fourier
144 method decomposing the distance of each point to the center of gravity of the outline (i.e. radius) as
145 a function of the distance along the outline. Each harmonic is weighted by two Fourier Coefficients
146 (FCs) using this method, reducing the dataset required to describe the outline. The zero harmonic A_0 ,
147 proportional to the outline size, was used as size estimator and to standardize all other FCs so they
148 represent shape variables only. The higher the rank of the harmonics, the more details they
149 represent on the outline. The shape of a mouse molar is adequately described by the first seven
150 harmonics, i.e. by 14 variables (Renaud et al. 2011).

151 **Statistics and multivariate analysis of the outline data**

152 Size differences were tested by an ANOVA and associated pairwise Tukey tests, the size estimator
153 being A_0 . Shape differences were tested by a Multivariate Analysis of Variance (MANOVA).

154 The pattern of shape difference between groups was assessed using a between-group Principal
155 Component Analysis (bgPCA). The total variance covariance (VCV) matrix \mathbf{T} is decomposed into the
156 between-group matrix \mathbf{B} and the within-group matrix \mathbf{W} , with $\mathbf{W} = \mathbf{T} - \mathbf{B}$. The bgPCA corresponds to
157 the eigenanalysis of \mathbf{B} . This analysis also provides the percentage of between-group relative to total
158 variance.

159 The amount of shape variance per group was assessed as the sum of the variance of the 14 FCs.

160 **Transgression in hybrids**

161 The degree of transgression was assessed as the deviation of the hybrids from the theoretic
162 expectations of them being perfectly intermediate between parents, hence for F1 hybrids: $d(F1,$
163 $WLA) + d(F1, PWK) - d(WLA, PWK)$ expressed as a percentage of the inter-parental strains distance
164 $d(WLA, PWK)$ (Renaud et al. 2012). Individual level of transgression was estimated in the same way,
165 based on the Euclidean distances of a descendent to the means of both parental groups.

166 **Measure of morphological integration ('integratedness')**

167 The variance of the eigenvalues of the VCV matrix has been proposed as a measure of phenotypic
168 integration (Pavlicev et al. 2009). If a complex trait varies in an integrated way, the signal will be
169 decomposed on few important eigenvalues λ_j and many of low importance, hence leading to a high
170 variance of the eigenvalues. The morphological integration (or 'integratedness', to distinguish this
171 realized integration from the potential of the developmental system to produce integration
172 (Hallgrímsson et al. 2009)) was thus estimated as the variance of the eigenvalues scaled to the total
173 sample variance (the sum of the eigenvalues of the VCV matrix) (Jamniczky and Hallgrímsson 2011) of
174 the VCV matrix of the FCs for each group of the cross, as well as the three wild populations.

175 **Comparison of variance-covariance matrices**

176 The distance between each pair of VCV matrices was defined as one minus the matrix correlation
177 between the two. The matrix of distances obtained was subjected to a Principal Coordinate Analysis
178 (PCOA), in order to identify the existence of patterns of differences or clustering among VCV matrices
179 of each group (Jamniczky and Hallgrímsson 2011; Debat et al. 2006).

180 **Regional response of the tooth to hybridization**

181 It is difficult to reliably locate landmarks on the murine tooth because the most evident locations
182 (top of the cusps) are eroded by wear, leading to a pattern highly impacted by the age of the animals.
183 In front of this difficulty, we went back to the outline data, namely the 64 radii describing the shape
184 along the outline. The 64 radii were split into four subsets: the anterior (radii 1 to 7 and 58 to 64),
185 lingual (radii 8 to 25), posterior (radii 26 to 39), and labial (radii 40 to 57) parts (Fig. 1). The
186 boundaries between parts were anchored at radii displaying low inter-individual variance, and
187 chosen to balance the number of radii in each part (anterior = 14 radii; lingual = 18; posterior = 14;
188 labial = 18). Each radius is proportional to tooth size. The sum of the radii per tooth part provided an
189 estimate of the tooth size, by which all radii were standardized. This conserved the information
190 about the relative size and arrangement of each part relative to the others, thus corresponding to a
191 simultaneous fit in landmark-based approaches (Labonne et al. 2014; Klingenberg 2013).

192 Each data set was explored using a between-group PCA. Differentiation between parents, variance
193 and transgression were estimated for each part of the tooth. The differentiation between parents
194 was estimated (1) as the proportion of between-group variance in the bgPCA; and (2) as the sum of
195 the distances between parental means at each radius, divided by the number of radii composing
196 each part. Variance, differentiation (distance between parental means), and transgression (deviation
197 from the mean between parental groups, scaled to the mean parental value) were further estimated
198 at each radius.

199 The strength of the covariation between parts was assessed using RV coefficients, i.e. the sum of the
200 squared covariances between two sets of variables, divided by the total amount of variation in the
201 two sets of variables (Klingenberg 2009).

202 Multivariate statistics were performed using the R package ade4 (Dray and Dufour 2007).

203

204 **Results**

205 **Differences between laboratory hybrids and their parental strains**

206 **Molar size differences**

207 The two parental strains were very different in size, WLA having much larger teeth than PWK (Fig.
208 2A). A dominant effect of WLA was present in the offspring, BC-WLA and F1 having teeth of similar
209 size than WLA ($P > 0.05$). All other groups were different of each other.

210 **Molar shape differences**

211 Parental groups, hybrids and backcrosses were all different in shape ($P < 0.0001$).

212 The between-group differentiation represented 33.4% of the total variance between lab mice. The
213 parental strains were opposed along the first axis, explaining 83% of variance. F1 and F2 hybrids were
214 located just in the middle along this axis. Backcrosses were intermediate between F1s and F2s, and
215 the parental strain to which they were backcrossed (Fig. 2B). Along the second axis (9%), the hybrids
216 were shifted compared to both parental strains. This corresponded to a transgression in mean shape
217 of 12.8% for the F1 and 10.4% for the F2. Individual deviation from mean shape ('individual
218 transgression') was higher, especially in F2 (average individual transgression F1: 21.5%; F2: 47.6%).

219 **Variance in molar size and shape**

220 For size as for shape, variance was quite reduced in the parental strains and F1s (Fig. 3A,B). Variance
221 was increased in F2s, being more than 2.5 x greater than in F1s for size and 4.5 x for shape. Variance
222 in backcrosses was of intermediate levels. Levels of variance in backcrosses and F2s were comparable
223 to those observed in wild populations (Fig. 3A,B).

224 **Morphological integration**

225 The observed morphological integration ('integratedness') was estimated by the variance of the
226 eigenvalues (as % of the total inertia) of the PCA on each group. The higher the variance, the more
227 variance is explained by first few axes. Integratedness (Fig. 3C) tended to be higher in parental

228 groups, especially in PWK. Levels of integratedness were comparable in backcrosses and wild
229 populations. The lowest integratedness was displayed in F1s and F2s.

230 **Comparison of variance-covariance matrices**

231 The VCV matrices of F2 hybrids and backcrosses were close to wild populations (Fig. 4). In contrast,
232 the parental groups WLA and PWK diverged markedly from this cluster, PWK diverging along the first
233 PCO axis and WLA along the second one. The VCV structure of the F1s appeared intermediate
234 between the parental groups and the F2-BC-wild cluster. This pattern could be viewed as a hybrid
235 transgression for the VCV structure, echoing the transgression in shape.

236 **A regionalized response to hybridization**

237 The pattern of covariance between traits (hence, the structure of the VCV matrix and the associated
238 integratedness) is the result of several (co)variance-generating developmental processes. A change in
239 integratedness may be driven by changes in the respective variance of these processes (Hallgrímsson
240 and Lieberman 2008). These covariance-generating developmental processes may not impact equally
241 all regions of the tooth. We thus looked for the distribution of the response to hybridization along
242 the tooth. The variance along the outline appeared to be localized (Fig. 5), with high variance at the
243 anterior part, at the anterior lingual zone, at the posterior border of the tooth, and around the
244 anterior and posterior labial cusps. Between these variable zones were radii of low variance, where
245 we anchored boundaries between sets of radii that were used to investigate how the tooth varied
246 locally in response to hybridization.

247 The first axis of a bgPCA on these radii characterized the differentiation between parental groups, as
248 for the analysis of the FCs. The contribution of each radius to this axis allowed highlighting which
249 zone of the tooth contributed most to the inter-parental difference (Fig. 5). This difference
250 corresponded to an expansion of the anterior and posterior parts in PWK, opposed to less
251 pronounced labial cusps in WLA. The second axis mostly expressed transgression, involving a change
252 in the curvature of the antero-lingual zone, an expansion of the posterior part, but no major change
253 on the labial zone.

254 Looking at patterns of differentiation for each region of the tooth separately (Fig. 6), F1s and F2s
255 ranged from being almost perfectly intermediate between parental strains (labial region) to being
256 markedly transgressive (lingual region). Accordingly, the percentage of transgression varied
257 depending on the tooth part: very low for the labial part (F1: 2.3%, F2: 6.2%); intermediate for the
258 anterior and posterior parts (anterior: F1: 17.4%, F2: 13.8%; posterior: F1: 15.8%, F2: 8.9%) and high
259 for the lingual side (F1: 33.3%, F2: 20.5%). Transgression tended to increase when the amount of

260 between-group differentiation decreased (ratio of between- to total-variance: total 32.4%, anterior
261 36.5%, lingual 20.0%, posterior 28.4%, labial 43.8%). This estimate may be influenced by the amount
262 of variance in hybrids, though. We thus further estimated the differentiation between parental
263 groups only, as the ratio of between to total variance when considering WLA and PWK only. The
264 anterior and labial tooth parts displayed an important differentiation between parental lines
265 (anterior: 82.1%, labial: 83.4%) whereas the lingual and posterior parts were less differentiated
266 and/or more variable (lingual: 58.2%, posterior: 59.1%). The sum of the between-strains distances at
267 each radius, scaled to the number of radii composing each region of the tooth, provided a further
268 estimate of morphological differentiation, this time independent of the amount of intra-group
269 variation. The labial region appeared as almost twice as differentiated between parental strains as
270 any other tooth part (anterior: 0.0019; lingual: 0.0011; posterior: 0.0017; labial: 0.0033).

271 **Covariation between tooth parts**

272 The strength of the covariation between pairs of tooth regions was estimated using RV coefficients
273 (Table 1; Fig. 7). Overall, the regions of the tooth varied in a concerted way. Covariation was the
274 weakest between the lingual and labial part, and the highest between the anterior and lingual part.
275 The parental strains and the F1s tended to display low covariations; this may be due to their low
276 sample size and/or their low morphological variance, making difficult to evidence covariation.

277 **Summary of the results**

278 Moderate transgression was evidenced for overall tooth shape whereas tooth size displayed
279 dominance towards the largest parental size (WLA). The differentiation was unequally distributed on
280 the tooth, the parental strains differing mostly in their anterior and labial parts (Fig. 8). The
281 transgression was also distributed unequally, being almost absent in the labial part, and the highest
282 in the lingual part.

283 The structure of the VCV matrix itself evidenced hints of transgression, the hybrid VCV differing from
284 the parental matrices, and being close to wild-type variance-covariance. The two parental strains
285 displayed diverging VCV matrices. PWK, especially, displayed only a localized variance at the
286 posterior border of the tooth. WLA varied also on a localized manner, at its forepart, anterior lingual
287 part, posterior border, and posterior labial cusp. F2, in contrast, displayed a rather evenly distributed
288 variance all along the tooth.

289

290 **Discussion**

291 **Transgressive molar shape in hybrid mice**

292 Molar shape in hybrid mice appeared as mid-way between parental strains with moderate
293 transgression. This pattern was also found when decomposing the tooth in four regions (anterior,
294 lingual, posterior and labial). The level of transgression however varied, from almost absent in the
295 labial region to high in the opposite lingual region.

296 In terms of mean shape of the hybrids, the effect of hybridization was thus relatively similar all over
297 the tooth. In comparison, hybridization had a very modular effect on mandible shape, dominance for
298 one strain or another being found in some modules, while other parts displayed a shape mid-way of
299 both parents, with more or less transgression (Renaud et al. 2012). Furthermore, the different
300 regions of the tooth appeared to covary more together than did the modules of the mandibles. These
301 evidences are in agreement with the fact that the partition of the mandible was based on known
302 developmental and functional modules (Klingenberg and Navarro 2012; Monteiro et al. 2005),
303 whereas the parts of the tooth corresponded to spatio-temporal regions along the same
304 developmental cascade (Jernvall and Thesleff 2000). Modularity in response to hybridization was not
305 expected for the tooth as it was for the mandible, and indeed, it was not documented here.

306 Note that size reacted differently from shape. Tooth size displayed a clear dominance towards the
307 size of the largest parental strain. Size determinism involves genes such as growth factors that are
308 prone to impact very different traits of an organism, acting as an integrating factor across organs
309 (Klingenberg 2013; Renaud et al. 2012). Hence, a similar response of size to hybridization could have
310 been expected for different characters of the cranio-facial complex such as mandible and molar. The
311 mandible size of the two strains was however rather similar, hybrids displaying heterosis in mandible
312 size (Renaud et al. 2009). This underlines the potential for a decoupling between size and shape
313 evolution. Indeed, such a decoupling has been documented for some mice populations. Corsican
314 mice, for instance, displayed larger molars than their continental counterparts whereas their
315 mandibles were of similar size (Orsini and Cheylan 1988; Vigne et al. 1993).

316 **Differentiation from standing variation: impact on covariance generating processes**

317 Hybrids were not only transgressive in molar shape. They appeared to be more variable, and with a
318 different variance-covariance (VCV) pattern than their parents. The response to hybridization of the
319 molar tooth is thus in agreement with the relaxation of trait covariance (Selz et al. 2013) and a
320 reduction of the phenotypic integration (Parsons et al. 2011) hypothesized to promote evolvability in
321 hybrids. The level of variance and the VCV structure in hybrids, however, were closer to wild
322 populations than were the parental strains. A counterpoint of characterizing the effect of

323 hybridization is thus to question the mechanisms that led to the differences between parental
324 strains. These strains being inbred for many generations. The standing variation present in wild
325 populations presumably went partly lost, due to the random fixation of some alleles, leading to the
326 decreased variance observed in the parental strains. The observed variances and covariances are the
327 product of many variance and covariance-generating processes (Hallgrímsson et al. 2009), that may
328 have more or less localized effects (Mitteroecker and Bookstein 2008). The fixation of some alleles
329 should make silent some of these processes, leading to the fact that some regions of the tooth that
330 were variable in wild populations, and were variable in hybrids, were not variable in the parental
331 strains (Fig. 8). Since the loci that became fixed had no reasons to be the same in both strains, this
332 led to a divergence of the variance-covariance structure in each parental strain, away from the
333 ancestral structure and away from each other (Fig. 4). Finally, since less variance/covariance-
334 generating processes were expressed, this resulted in an increased morphological integration in the
335 parental strains. By restoring the ancestral standing variation, hybridization restored altogether the
336 wild-type level of variance and morphological integration as well as the VCV structure. This illustrates
337 on a simple model and for the mouse tooth what was observed in the cranium of various lab strains
338 vs. natural populations of rodents: lesser variance, higher morphological integration, distortion of
339 the covariance structure (Jamniczky and Hallgrímsson 2011). Such mechanisms could also be
340 responsible for changes in the variance-covariance structure occurring as a result of genetic drift,
341 especially in small populations (Whitlock et al. 2002).

342 **Regional variations in the degree of transgression: a link with the timing of development?**

343 Transgressive phenotypes in hybrids can arise from several genetic and epigenetic interactions. The
344 accumulation of genes with antagonistic effects in each parental group can produce transgressive
345 from the F2 generation (Rieseberg et al. 1999). Dominance at some loci may produce transgressive
346 phenotypes already in F1s (Stelkens and Seehausen 2009). In complex traits, epistasis can also
347 generate transgressive phenotypes. Such factors have no reason to vary along the developmental
348 cascade making the tooth. Yet, regional variations in the level of transgression were observed along
349 the outline of the tooth.

350 The labial part is the region showing both, the strongest divergence between parents and the almost
351 absence of transgression. The first cusp to be signaled in the developing molar lies in this area (Cho
352 et al. 2007). Few mutations may easily generate long-standing geometric differences early in the
353 cascade (Salazar-Ciudad and Jernvall 2006). In contrast, the lingual side displays little differentiation
354 between parents, but a high level of transgression. This is the last region of the tooth to develop (Cho
355 et al. 2007). Possibly, at this later moment of development, epigenetic interactions between the

356 tooth and neighboring tissues are more important, as suggested by complex interactions between
357 many genetic networks at this stage (Landin et al. 2015). Such epistatic interactions are prone to
358 generate transgression (Brem and Kruglyak 2005; Stelkens and Seehausen 2009). In contrast, few
359 mutations may have lesser geometric effect late in development than early: all other cusps being
360 already determined, they should constrain the range of spatial variation left to the last forming
361 region.

362 The anterior part of the tooth appeared to be in an intermediate situation, with an intermediate
363 differentiation between parents, but a relatively important transgression. This region of the tooth
364 develops relatively late, slightly before the lingual side, which may explain their high covariance due
365 to a shared late formation, and common neighboring influences. But it is also a region that is
366 patterned early during development, with the expression of vestigial buds anterior to the molar-
367 developing zone (Renaud et al. 2011; Peterkova et al. 1996). This may illustrate that differentiation
368 may accumulate more easily in tooth regions patterned early in the cascade, whereas transgression
369 would be favored in late-developing regions, prone to many epigenetic influences of other
370 developing organs such as the surrounding jaw. This challenges further studies exploring the
371 consequence of a developmental cascade on the genotype-phenotype map (Salazar-Ciudad and
372 Marin-Riera 2013).

373 **A link between differentiation and transgression?**

374 The amount of transgression has been evidenced to be negatively correlated to the amount of
375 genetic divergence between hybridizing taxa in a set of animal models (Stelkens & Seehausen 2009).
376 Within the tooth, a similar relationship seems to emerge, the most differentiated region being the
377 less transgressive. First, some transgression may occur in any case of hybridization, but it will tend to
378 appear more important in case of low morphological differentiation between parents, because for
379 comparison purpose, transgression is most often expressed in percentage of the inter-parental
380 distance. A same amount of transgressive shift will thus be expressed as a higher percentage of
381 transgression if the parental strains are less differentiated.

382 Second (and not mutually exclusive), a link between the processes underlying the inter-parental
383 differentiation and the hybrid transgression may exist. Many complementary genes and epistasis
384 may tend to impede phenotypic differentiation but promote transgression. Further investigations are
385 required to test for this link, with the problem of comparing levels of differentiation, transgression
386 and variation in different cases of hybridization and different morphological characters.

387 Noteworthy, mandible and molar of the same hybrid mice achieved a similar level of transgression
388 despite obvious differences in their developmental properties. They apparently did so, however,
389 following different processes. Modular response to hybridization contributed to the generation of
390 novel phenotypes for the mandible. In contrast, the inbreeding in the two parental strains altered the
391 expression of some major (co)variation generating processes in the molar, that were restored in
392 hybrids. Important epistasis may have further promoted transgression, but the importance of this
393 factor seems to vary along the spatio-temporal development of the tooth.

394

395 **Conclusions**

396 Hybridization is more and more advocated to be a fuel for evolution, increasing evolvability by
397 increasing the standing variation and lowering phenotypic integration (Parsons et al. 2011; Selz et al.
398 2013), thus offering new possibilities to the screening of selection (Nolte and Sheets 2005; Nichols et
399 al. 2014). In agreement with these expectations, hybrid mice displayed here an increased variation in
400 molar shape, a decreased phenotypic integration and an altered structure of variance-covariance. In
401 counterpoint, the inbred parental strains documented the effect of a loss of standing genetic
402 variation causing changes in the variance-covariance structure, and the expression of (co)variation
403 generating processes. The effects of these processes seem to vary regionally on the tooth, possibly
404 depending on the developmental dynamics. The reshuffling of the standing genetic variation in
405 hybrids should have restored the expression of all (co)variation generating processes. The potential
406 for morphological novelties will depend on the degree of divergence reached by the parental groups,
407 compared to the ancestral standing genetic variation. Considering inbred strains may have provided
408 here a situation analogue to a higher degree of divergence than actually occurring in field hybrids of
409 the two subspecies of the house mouse.

410

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416 ~~XXX~~.

417

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541

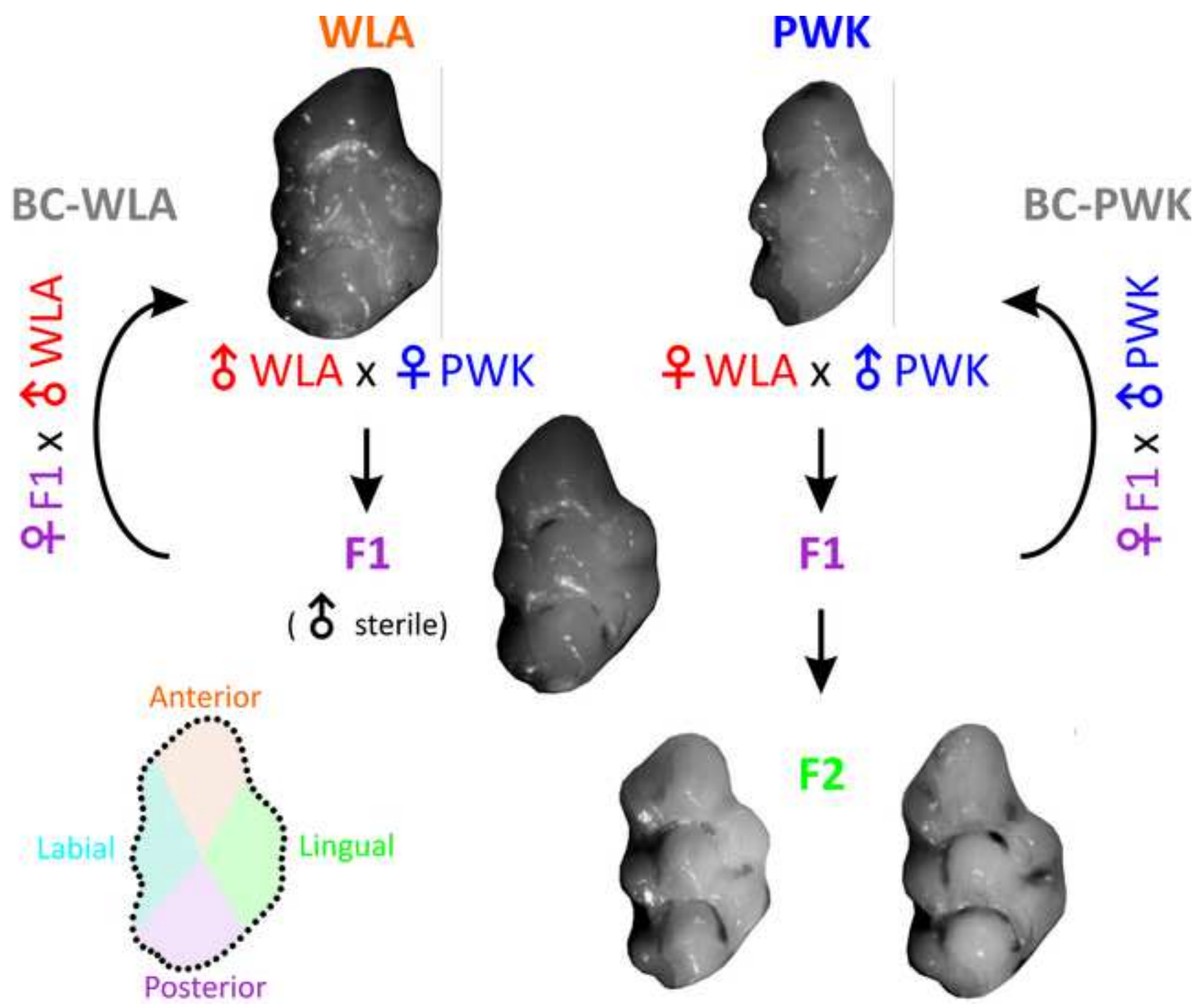
	Ant/Ling		Ant/Post		Ant/Lab		Ling/Post		Ling/Lab		Post/Lab	
	RV	P	RV	P	RV	P	RV	P	RV	P	RV	P
WLA	0.384	0.000	0.360	0.000	0.309	0.001	0.300	0.001	0.484	0.000	0.476	0.000
PWK	0.312	0.003	0.244	0.013	0.371	0.001	0.683	0.000	0.496	0.000	0.408	0.001
F1	0.432	0.000	0.153	0.041	0.274	0.000	0.262	0.001	0.291	0.001	0.307	0.001
F2	0.655	0.000	0.432	0.000	0.332	0.000	0.326	0.000	0.258	0.000	0.374	0.000
BC-WLA	0.756	0.000	0.558	0.000	0.411	0.000	0.404	0.000	0.177	0.000	0.433	0.000
BC-PWK	0.532	0.000	0.433	0.000	0.506	0.000	0.278	0.000	0.276	0.000	0.428	0.000
Gardouch	0.485	0.000	0.367	0.000	0.424	0.000	0.228	0.000	0.133	0.004	0.374	0.000
Marion	0.559	0.000	0.489	0.000	0.454	0.000	0.220	0.000	0.192	0.000	0.483	0.000
Fango	0.806	0.000	0.602	0.000	0.441	0.000	0.415	0.000	0.182	0.002	0.529	0.000

542

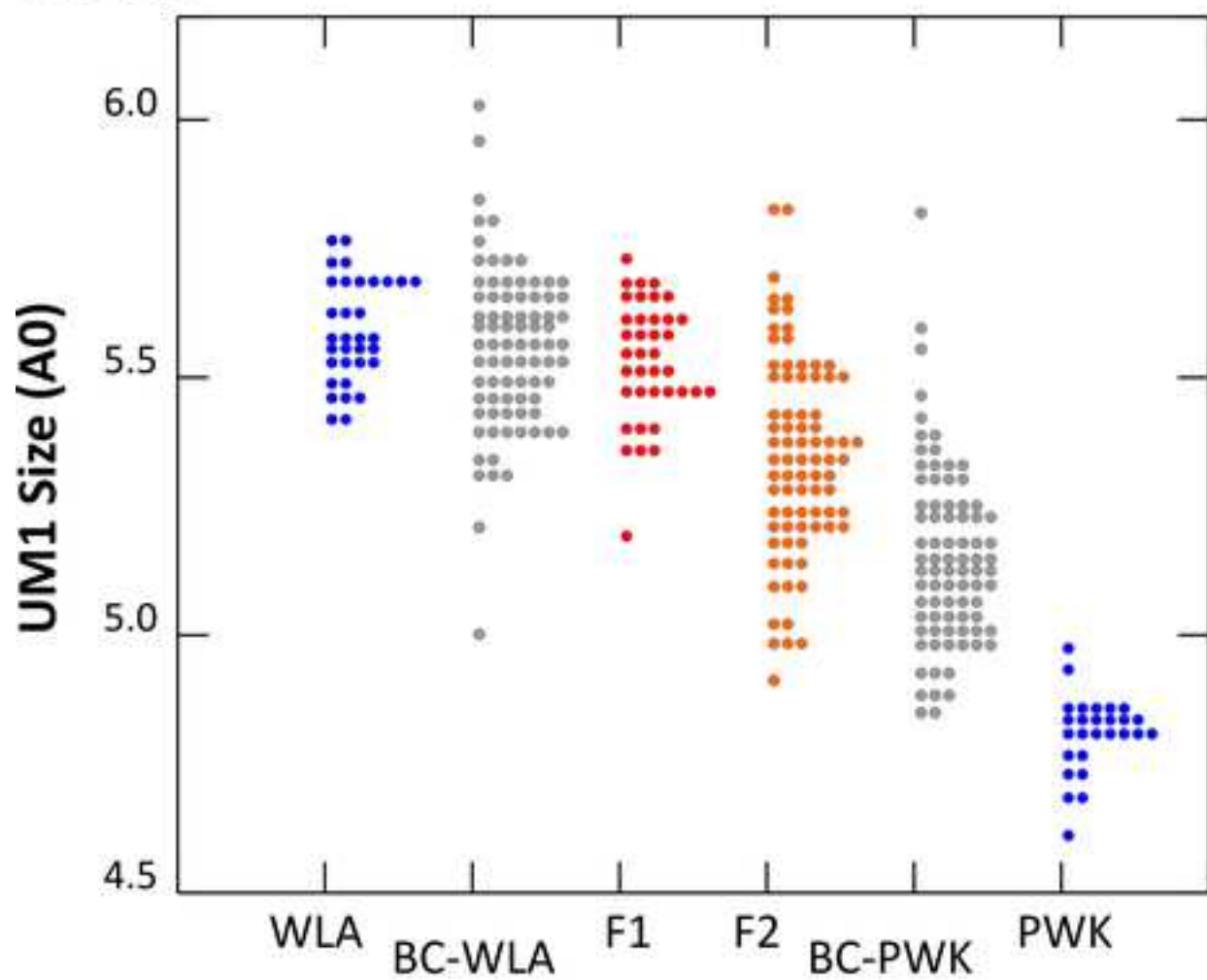
543 **Table 1.** Integration between tooth part. RV coefficients are provided, together with their
544 probabilities (observed value vs. 9999 permutations). In bold probabilities ≤ 0.001 .

545

546



A. Size



B. Shape



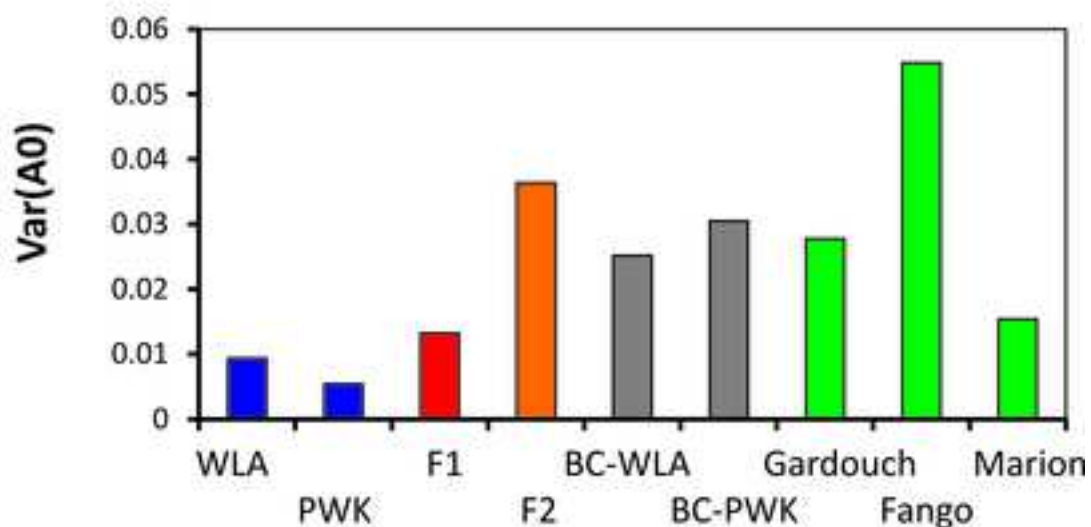
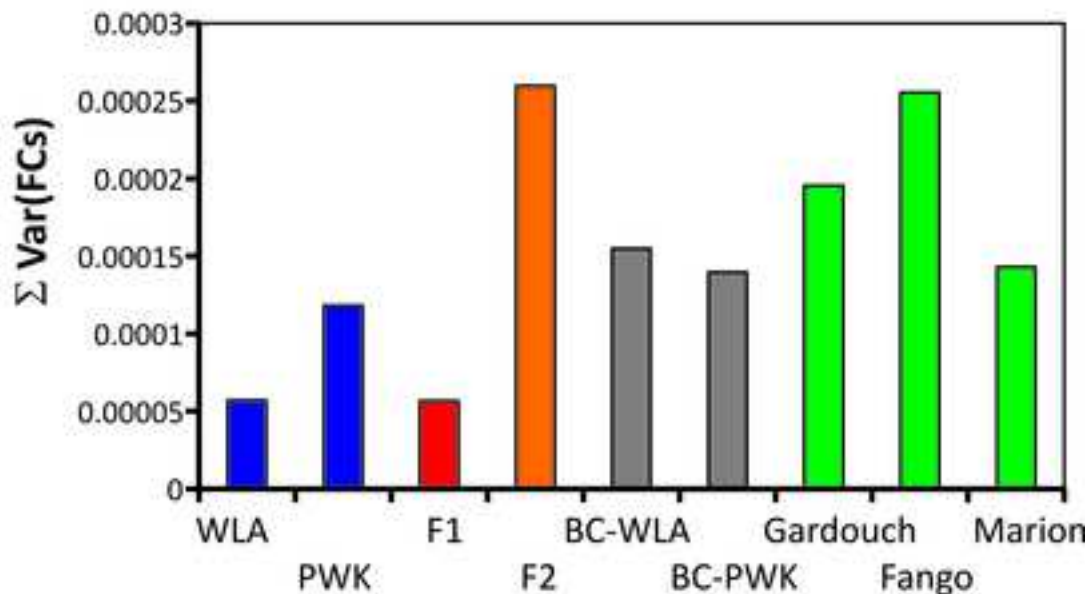
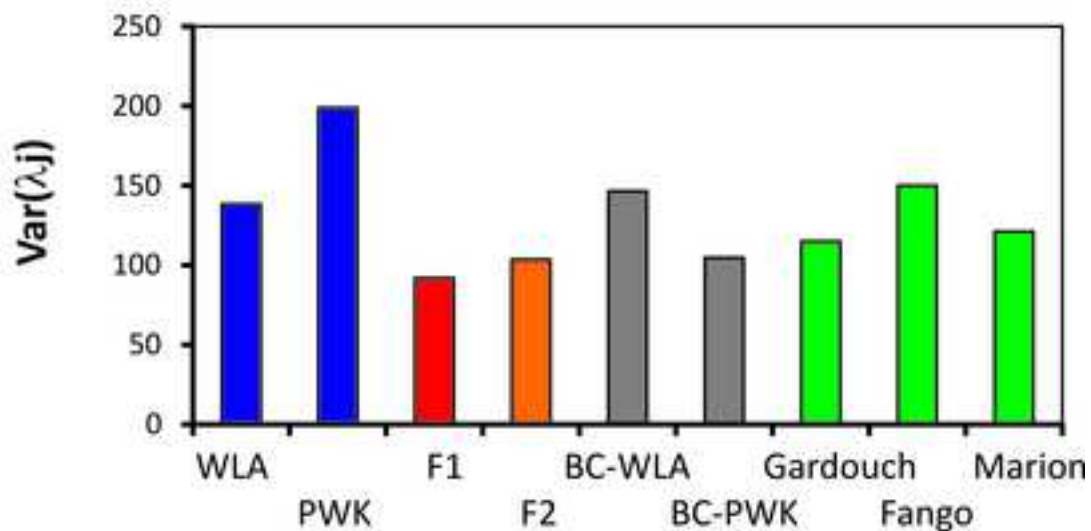
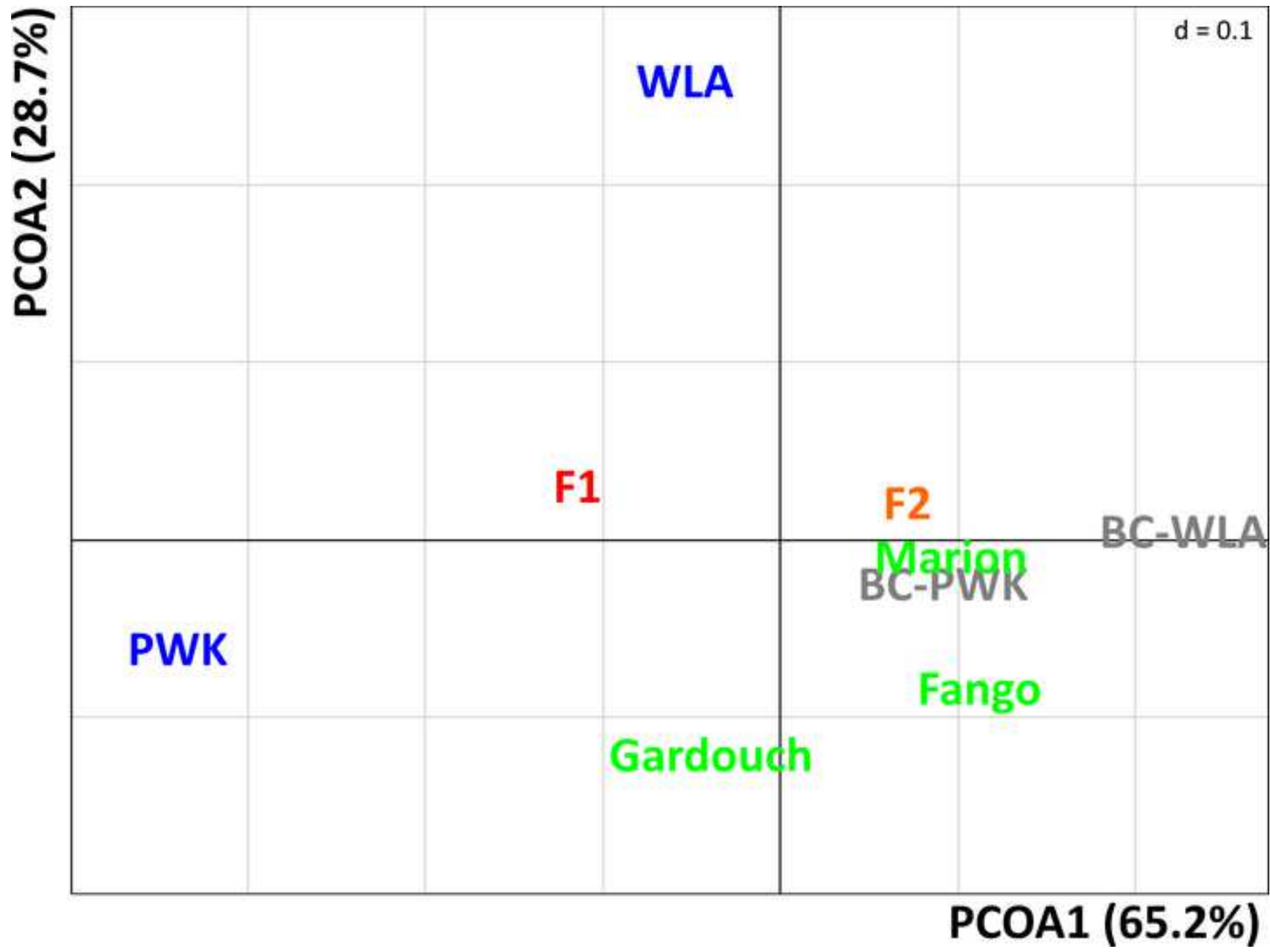
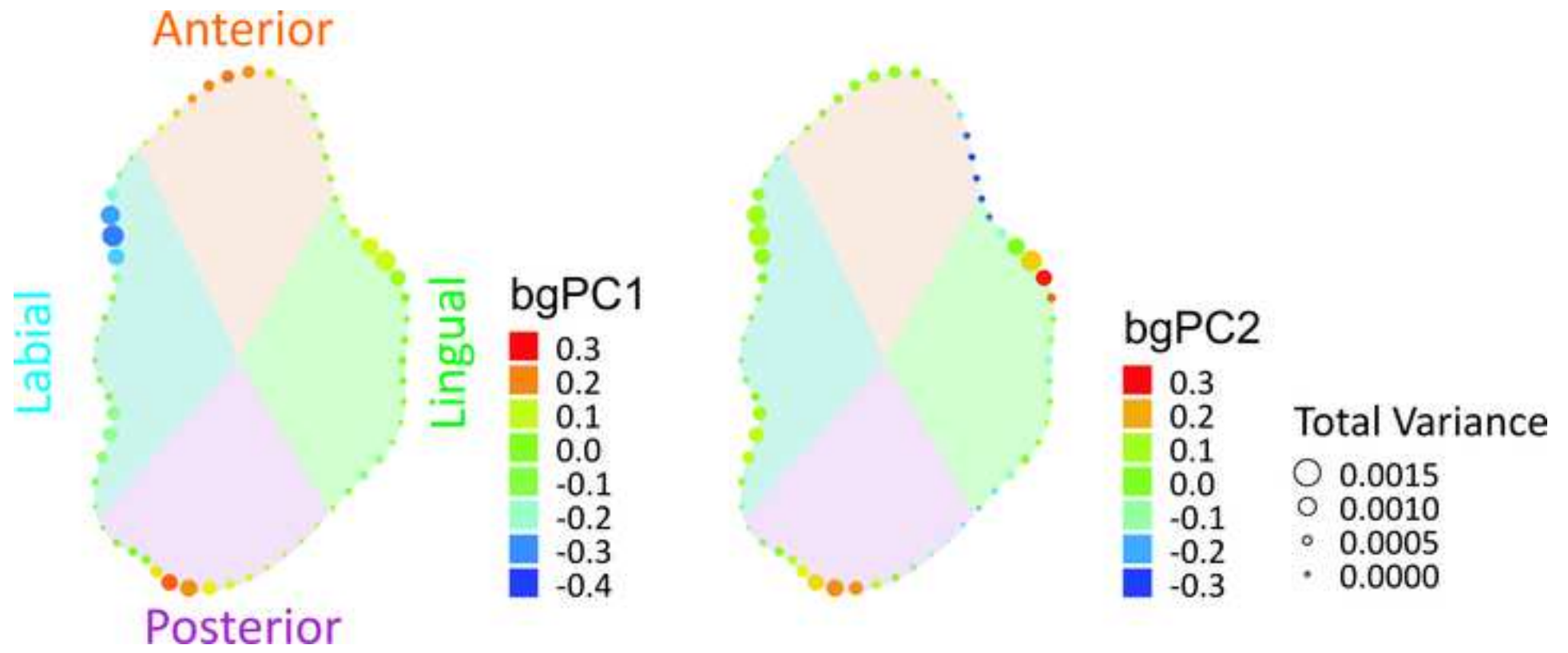
A. Size Variance**B. Shape Variance****C. Integratedness**

Figure 4





A. Anterior Part - bg 36.5%



B. Lingual Side - bg 20.0%



C. Posterior Part - bg 28.4%



D. Labial Side - bg 43.8%

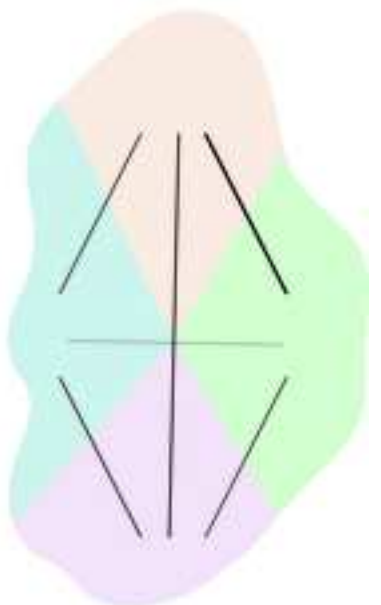
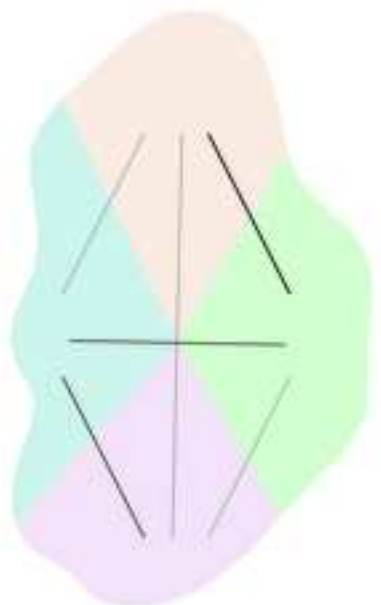
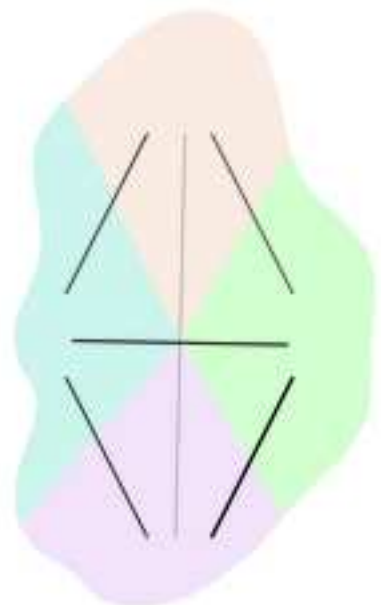
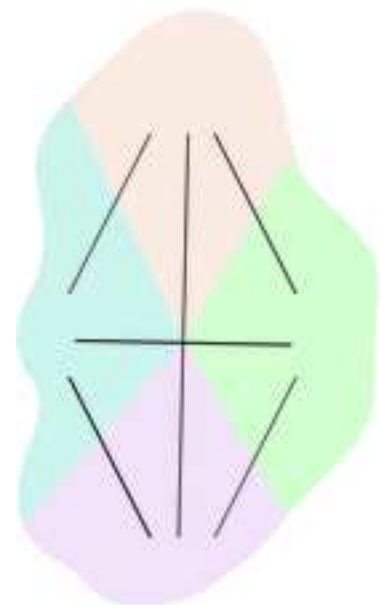


WLA

PWK

F1

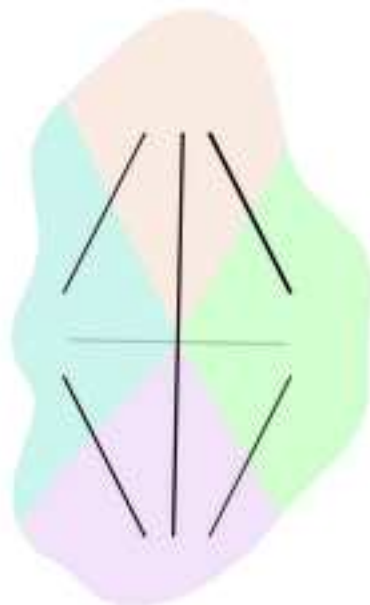
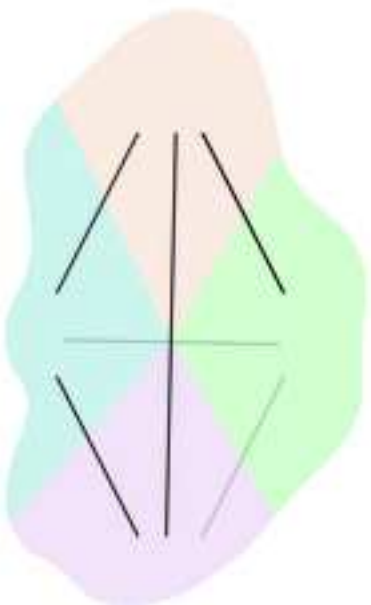
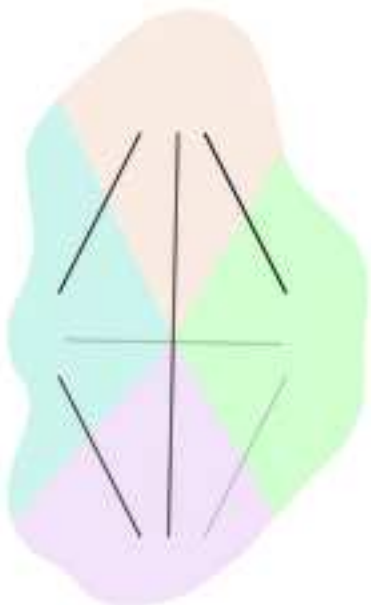
F2



Gardouch

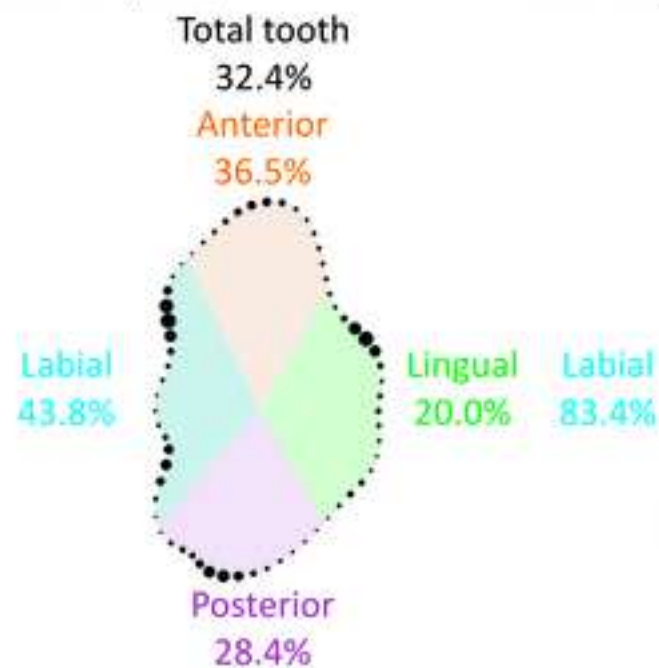
Marion

Fango

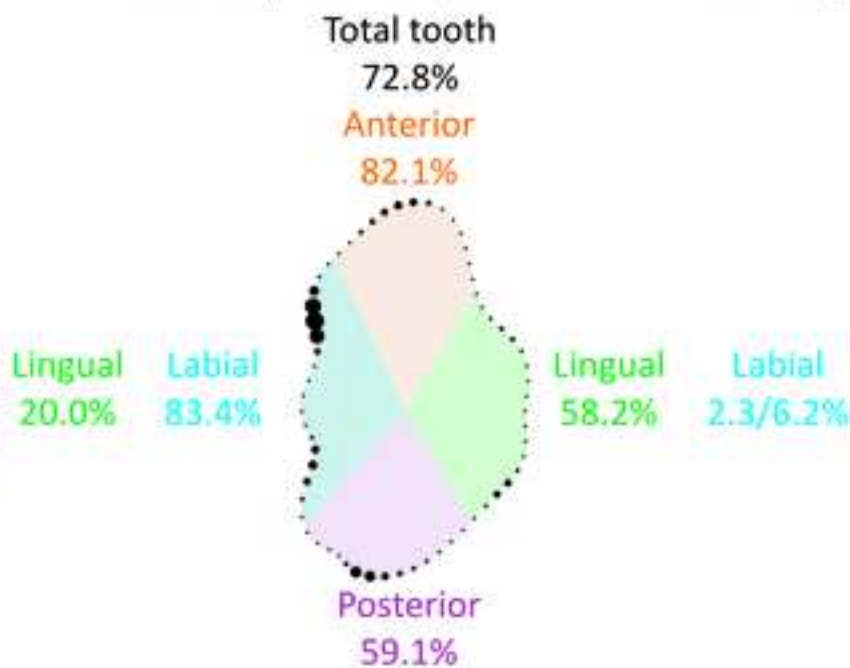


A. bg-ratio all groups

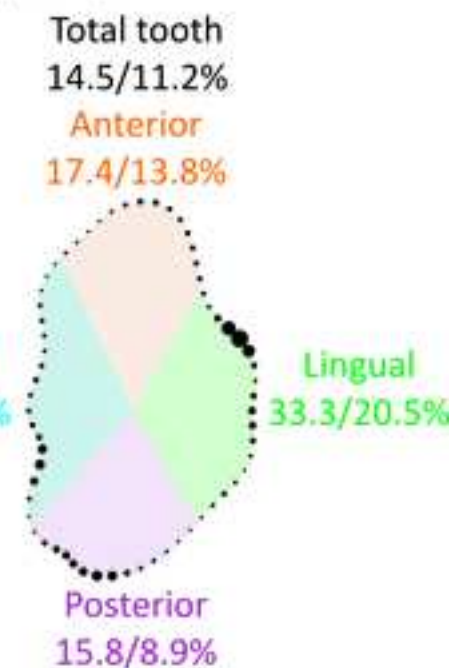
size: var(total)

**B. bg-ratio parents**

size: var(WLA+PWK)

**C. Transgression F1/F2**

size: var(F1+F2)

**D. WLA**size: var(WLA)
color: Mean-WLA**E. PWK**size: var(PWK)
color: Mean-PKW**F. F1**size: var(F1)
color: Transgression**G. F2**size: var(F2)
color: Transgression