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

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

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Abstract

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 (*Mus musculus domesticus* and *M. m. musculus*). 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.

Keywords

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

Introduction

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

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

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

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

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

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

Material and Methods

Material

Laboratory hybrid mice

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

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

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

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

Wild-trapped mice

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

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

Methods

Outline analysis

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

trigonometric functions of decreasing wavelength (harmonics) by a Fourier approach. Each harmonic is weighted by four Fourier coefficients (FCs), two for x- and two for y-variations. This method allows the alignment of the outline along the first axis of the ellipse best fitting the outline, and the adjustment of the starting point at the intersection of the outline with this first axis, corresponding to the major elongation of the object. A drawback is a high number of variables required to describe the outline (four FCs by harmonics). This method was used to reconstruct an outline with the starting point adjusted along the first axis of the major ellipse. This outline was then analyzed using a Fourier method decomposing the distance of each point to the center of gravity of the outline (i.e. radius) as a function of the distance along the outline. Each harmonic is weighted by two Fourier Coefficients (FCs) using this method, reducing the dataset required to describe the outline. The zero harmonic A0, proportional to the outline size, was used as size estimator and to standardize all other FCs so they represent shape variables only. The higher the rank of the harmonics, the more details they represent on the outline. The shape of a mouse molar is adequately described by the first seven harmonics, i.e. by 14 variables (Renaud et al. 2011).

Statistics and multivariate analysis of the outline data

Size differences were tested by an ANOVA and associated pairwise Tukey tests, the size estimator being A0. Shape differences were tested by a Multivariate Analysis of Variance (MANOVA).

The pattern of shape difference between groups was assessed using a between-group Principal Component Analysis (bgPCA). The total variance covariance (VCV) matrix **T** is decomposed into the between-group matrix **B** and the within-group matrix **W**, with **W = T – B**. The bgPCA corresponds to the eigenanalysis of **B**. This analysis also provides the percentage of between-group relative to total variance.

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

Transgression in hybrids

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

Measure of morphological integration ('integratedness')

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

Comparison of variance-covariance matrices

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

Regional response of the tooth to hybridization

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

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

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

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

Results

Differences between laboratory hybrids and their parental strains

Molar size differences

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

Molar shape differences

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

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

Variance in molar size and shape

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

Morphological integration

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

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

Comparison of variance-covariance matrices

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

A regionalized response to hybridization

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

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

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

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

Covariation between tooth parts

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

Summary of the results

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

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

Discussion

Transgressive molar shape in hybrid mice

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

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

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

Differentiation from standing variation: impact on covariance generating processes

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

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

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

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

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

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

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

A link between differentiation and transgression?

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

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

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

Conclusions

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

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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**

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

Figure 1

Click here to download Figure Fig1_Cross_simplified_symb..jpg

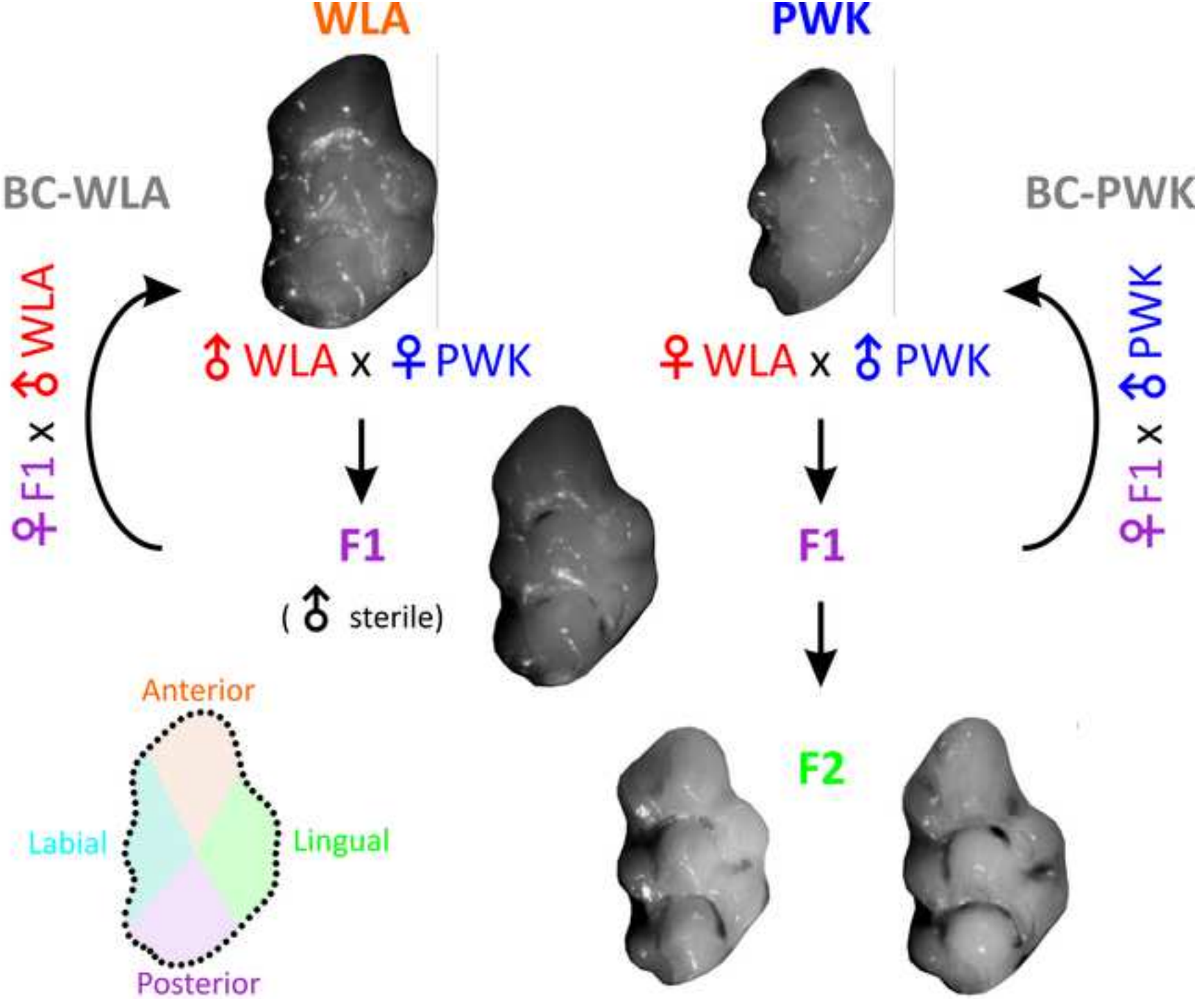

Figure 2

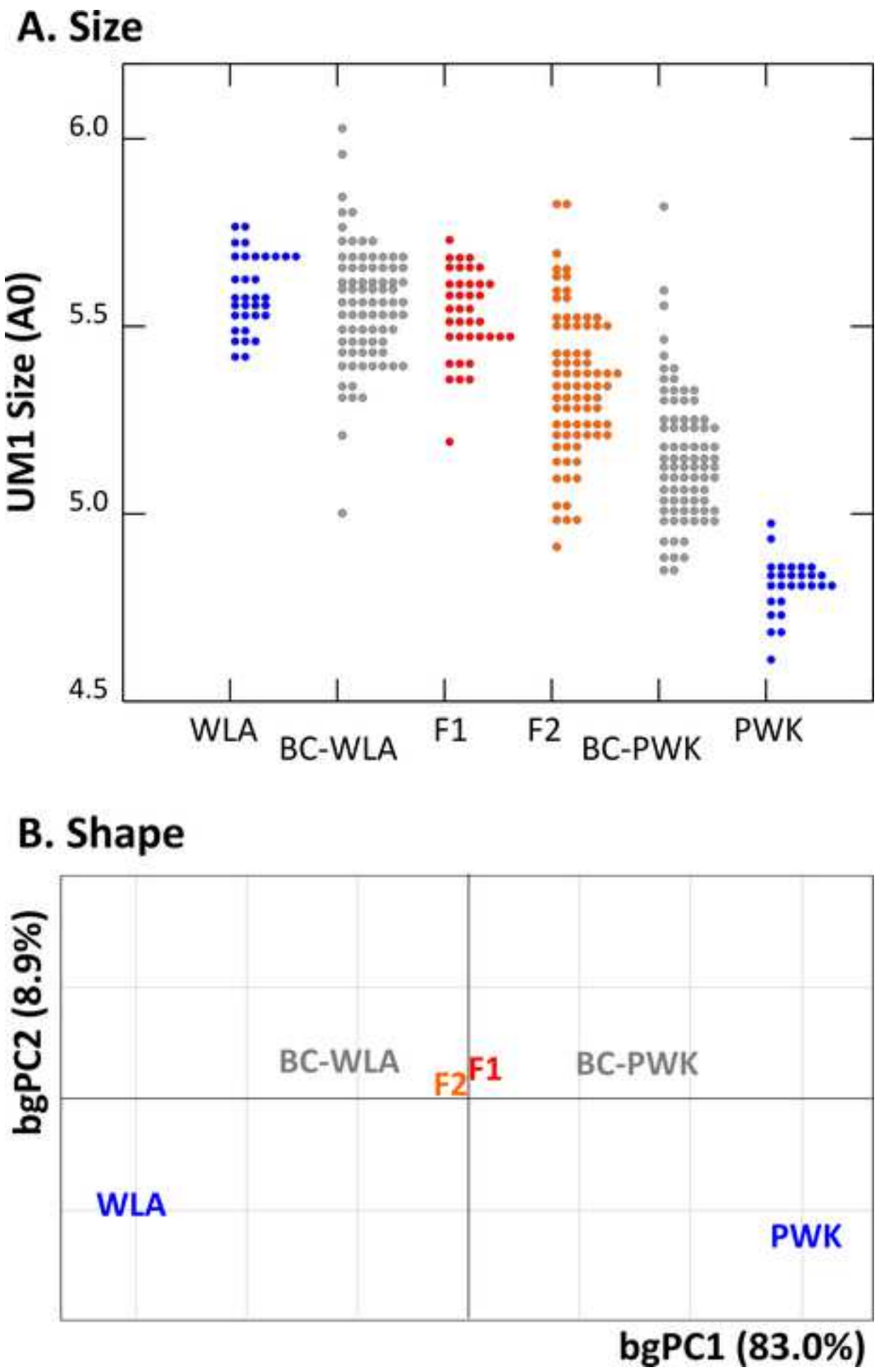

Figure 3

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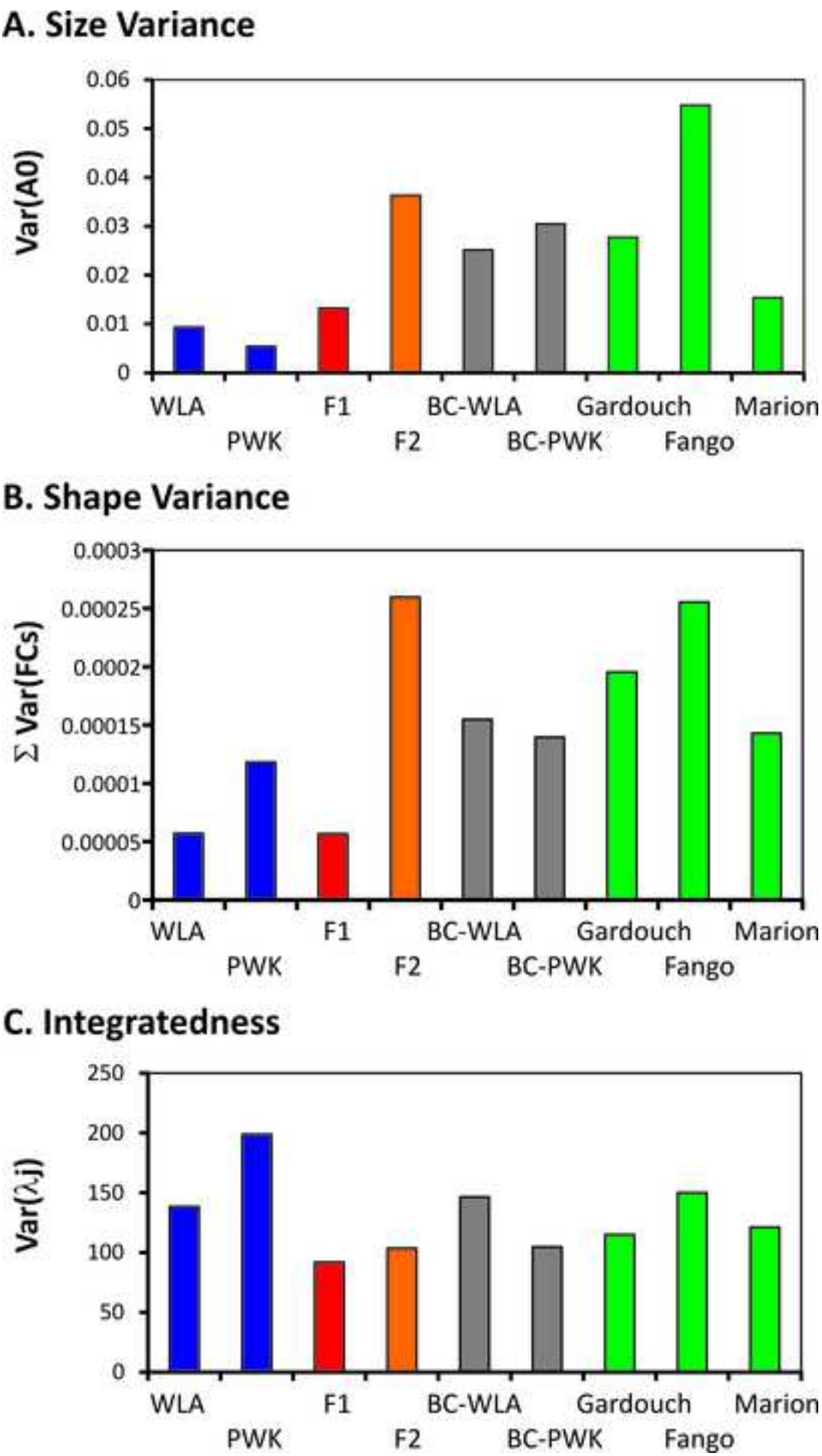

Figure 4

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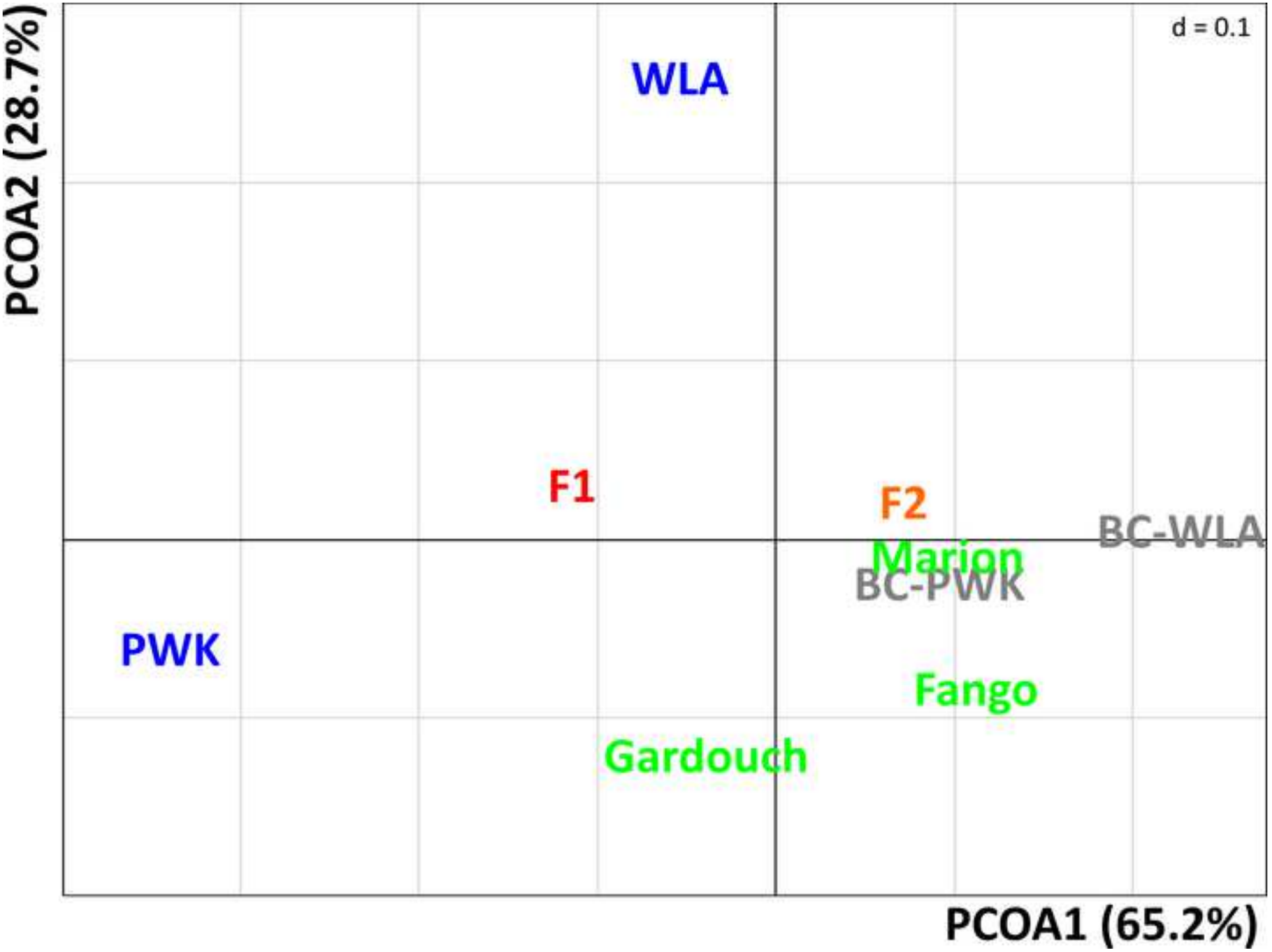

Figure 5

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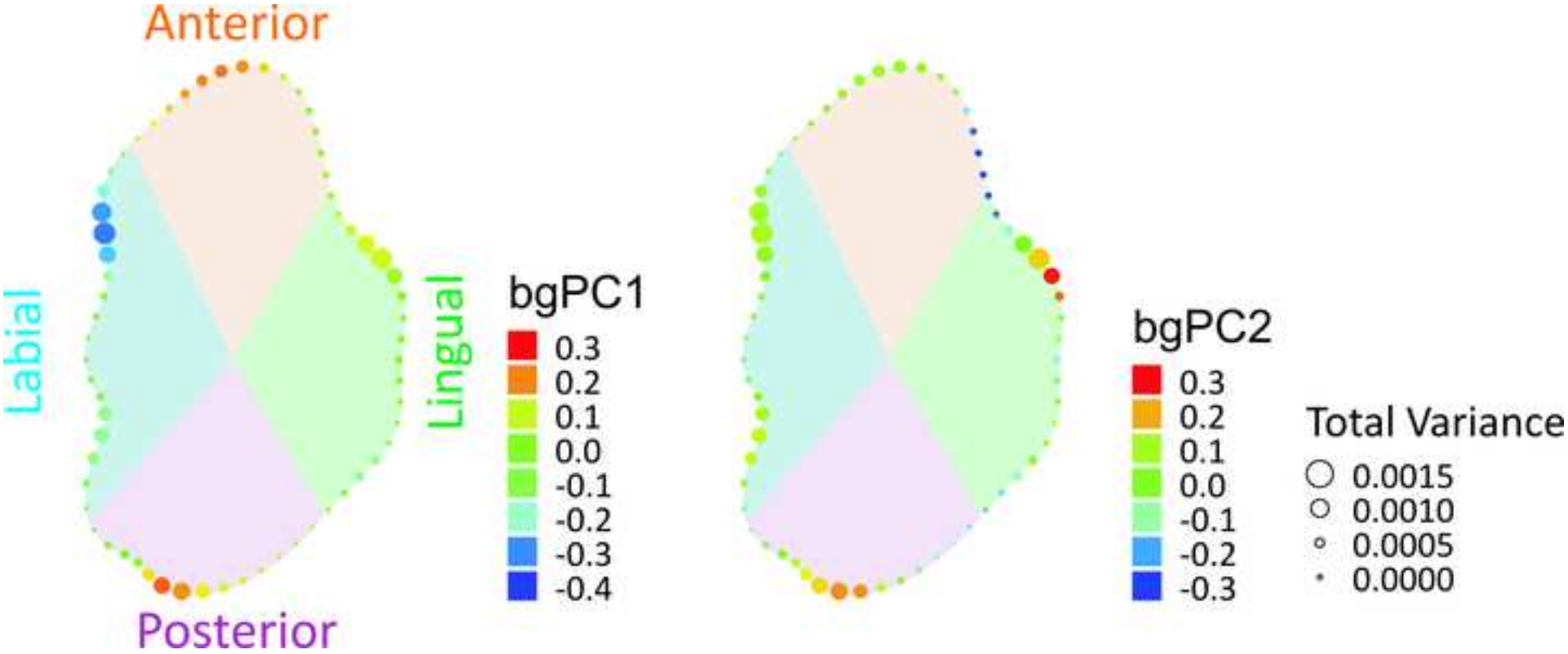

Figure 6

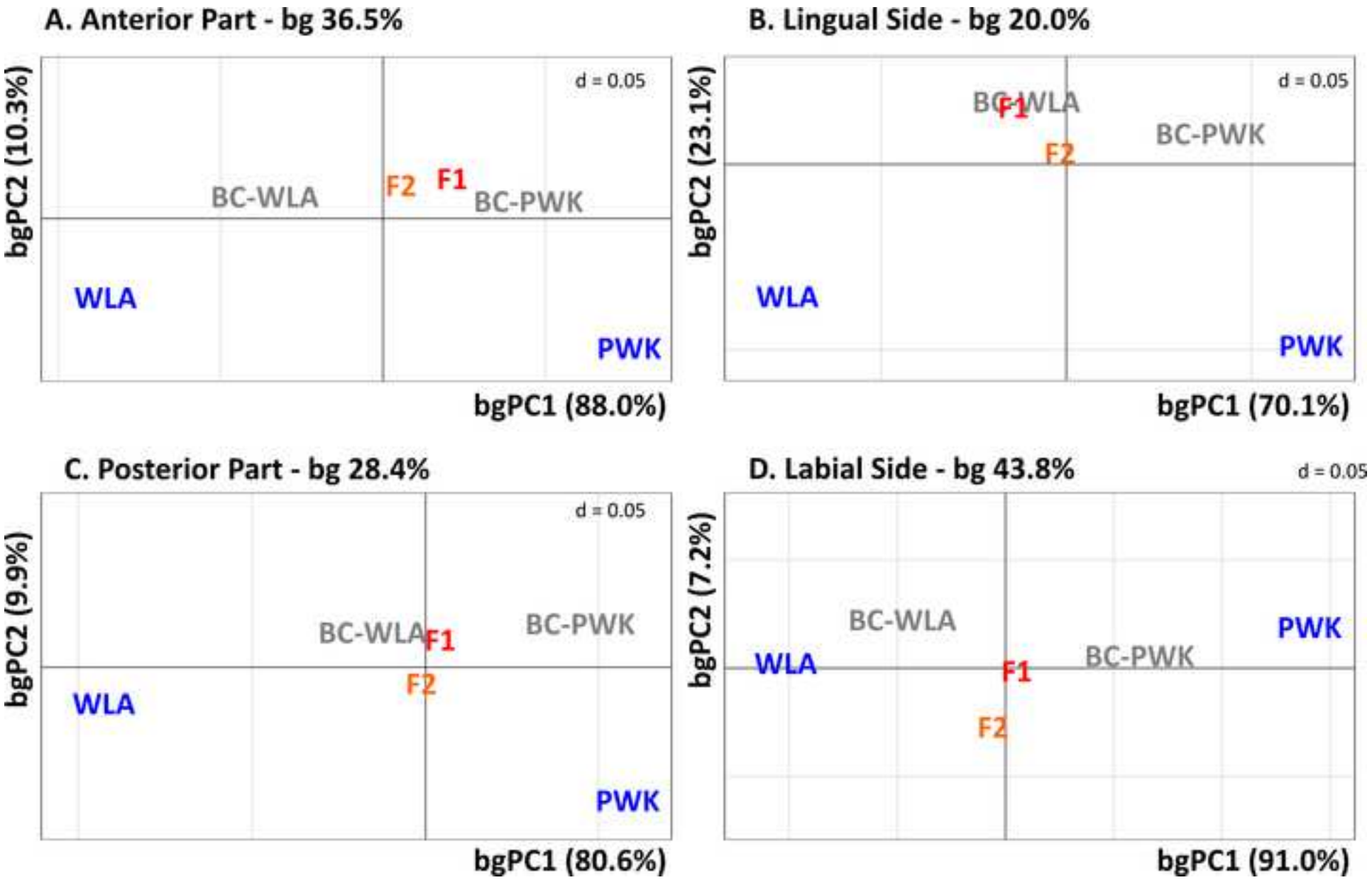

Figure 7

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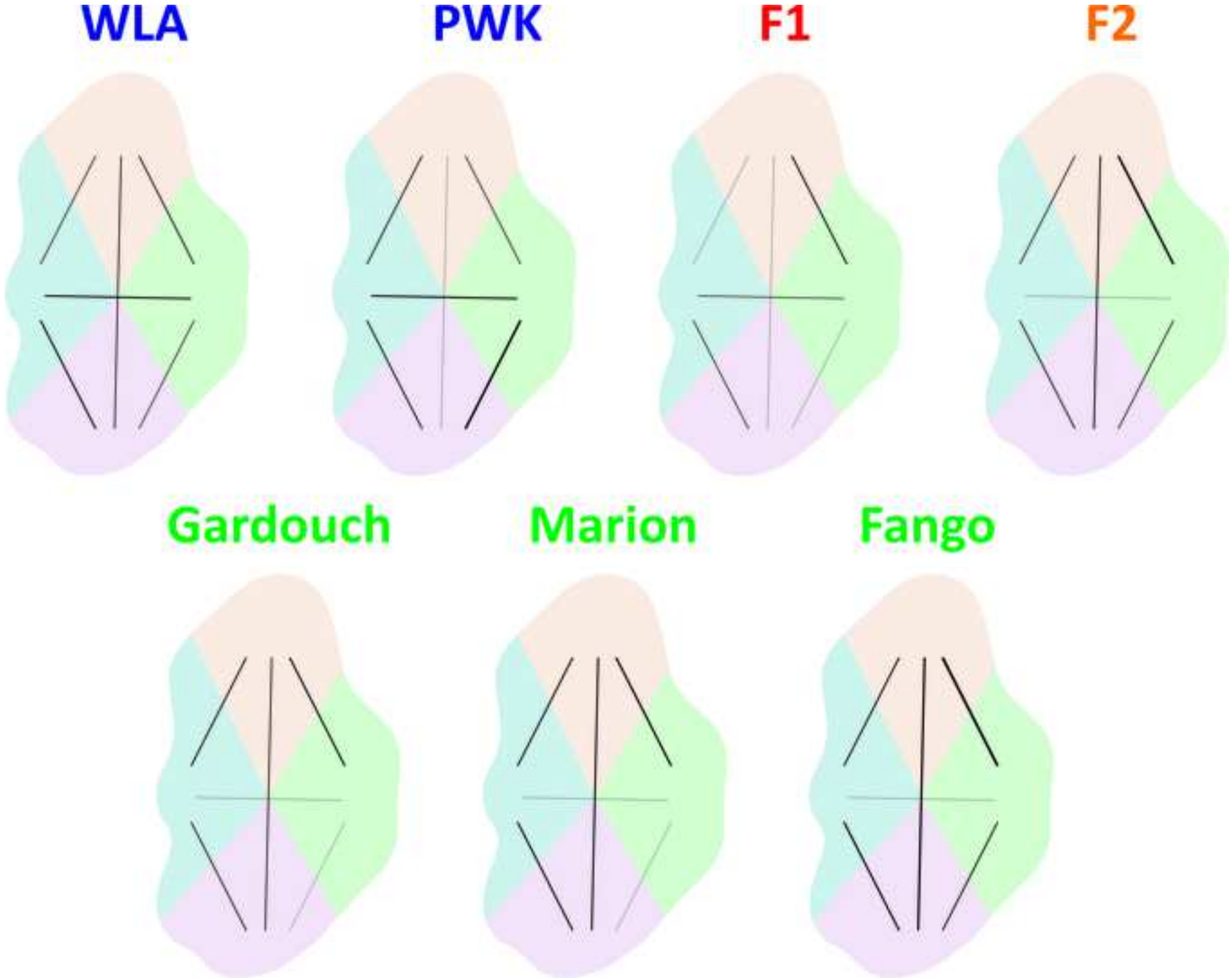

Figure 8