Craniofacial diversification in the domestic pigeon and the evolution of the avian skull

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A central question in evolutionary developmental biology is how highly conserved developmental systems can generate the remarkable phenotypic diversity observed among distantly related species. In part, this paradox reflects our limited knowledge about the potential for species to both respond to selection and generate novel variation. Consequently, the developmental links between small-scale microevolutionary variations within populations to larger macroevolutionary patterns among species remain unbridged. Domesticated species, such as the pigeon, are unique resources for addressing this question, because a history of strong artificial selection has significantly increased morphological diversity, offering a direct comparison of the developmental potential of a single species to broader evolutionary patterns. Here, we demonstrate that patterns of variation and covariation within and between the face and braincase in domesticated breeds of the pigeon are predictive of avian cranial evolution. These results indicate that selection on variation generated by a conserved developmental system is sufficient to explain the evolution of crania as different in shape as the albatross or eagle, parakeet or hummingbird. These ‘rules’ of craniofacial variation are a common pattern in the evolution of a broad diversity of vertebrate species and may ultimately reflect structural limitations of a shared embryonic bauplan on functional variation.

Darwin wrote: “We may look in vain through the 288 known species [of pigeons and doves] for a beak so small and conical as that of the short-faced tumbler; for one so broad and short as that of the barb; for one so long, straight, and narrow … as that of the English carrier…”

Domesticated animals have long played an important role in our understanding of the evolution and diversification of natural species. One of Darwin’s key insights was that domesticates provided an analogous and accelerated window into the vastly slower process of natural selection, accomplishing in generations what may normally take millions of years. One species in particular, the common pigeon, was central to his understanding of selection and its role in evolution. Among other traits1–4, Darwin noted how the shape of the pigeon’s head and beak has been subjected to extensive selection by humans5, resulting in breeds that qualitatively not only exceed variation seen within a single wild bird species, but also converge on phylogenetically distant avians (Fig. 1 and Supplementary Fig. 1).

Darwin believed selection was the primary driving breeding species diversification, but he also observed how the “correlation of growth among body parts” could confound the breeder’s goals5–7. That said, he was largely unaware of how these rules were determined or how they might ultimately impact phenotypic diversification. In the modern view, genetic pleiotropy drives covariation8–10 through the correlated effects of variation in developmental processes4, in turn biasing the long-term direction and magnitude of evolutionary responses to selection10. However, there is continued debate about the relative importance of development and selection to the patterning of macroevolutionary diversity, since evolutionary changes can occur in both the distribution of variation across such processes and in the developmental basis for the correlations themselves8. Consequently, selection not only impacts observed phenotypic distributions, but also can alter the rules for how variation is generated within the ‘space’ in which phenotypes exist11,12.

In this context, the dramatic variation among domesticated pigeon breeds provides critical evidence of not only selection, but also a unique window into how the developmental potential of a single species corresponds to evolutionary diversification. Avian diversity is driven by a myriad of functional factors that are particular to lineages and contingent within the history of each group. If the diversity of morphology generated within a single bird species were to resemble that produced by evolution among all birds, then this would provide powerful evidence for the existence of key developmental drivers of correlations among parts that persist across broad ranges of phylogenetic diversity. Despite the potential importance of domestic breeds to these questions, there have been few attempts to either characterize skeletal variation in the pigeon13, or relate them to broader avian radiations14. Here, we test whether differences in morphological variation among avians and domestic and feral pigeon populations reflect changes in the organization of cranial covariation, measured in terms of pattern and magnitude of integration and modularity, or if instead variation within and among these groups reflects the potential of a common developmental system to generate phenotypic diversity.

Results

We first compared feral and domestic pigeons to proxies of the wild-type ancestral populations, the rock dove. Linear data for rock doves and feral and domestic pigeons (Supplementary Table 1) were significantly correlated with overall body size. All groups overlapped and there were no significant differences in slope, with the exception of premaxilla and cranial width in domestic (Supplementary Fig. 2). In all dimensions, domestices exhibited increased mean and variance

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(Levene’s test, $P < 0.0001$), with the average coefficient of variation (c.v.) across traits threefold greater than estimated for feral populations (c.v.$_{\text{dom}} = 13.3\%$, c.v.$_{\text{fer}} = 4.5\%$) and nearly fourfold that of the wild rock dove (c.v.$_{\text{rrd}} = 3.6\%$) (Supplementary Table 2). Ferals were more variable in some traits (premaxilla and skull width, overall size) relative to the rock dove (Levene’s test, $P < 0.05$), but not others (premaxilla and mandible length; Levene’s test, $P > 0.05$), in part due to geographic population heterogeneity. Increased variation in domestics was generally consistent with predictions of the rock dove within-group regression, with the exception of width. The first axis of the principal component analysis (PCA) (PC1, 81.5\% variation) was significantly associated with size (geometric mean) ($r^2 = 0.958$, $P < 0.0001$), with no significant difference in slope among groups ($P = 0.522$) (Fig. 2). The pattern of trait correlations among pigeons was highly significant when adjusted for repeatability (matrix correlation ($r_m$) > 1 between rock dove and domestics ($P < 0.0001$); $r_m = 0.798$ for the feral–domestic comparison ($P < 0.0001$); Supplementary Table 3). The unadjusted variance of the eigenvalues (v.e.) of the domestics was significantly higher than wild doves and pigeons, but was statistically indistinguishable from the rock dove at comparable c.v. values (for example, when c.v.$ = 3$, v.e.$_{\text{rrd}} = 0.56$ and v.e.$_{\text{dom}} = 0.62$) and converged with all wild types when c.v.$ = 6$. Together, these results suggest that domestics have increased magnitude of variation produced by a trait correlation structure shared with wild pigeons.

Next, we turned to the pigeon shape data. Here, brain–shape allometry accounted for 20.2\% of total variation ($P < 0.001$), with smaller individuals having proportionately shorter, straighter beaks and wider crania (Supplementary Fig. 3). The first three principal components of non-allometric pigeon cranial shape variation described facial length (PC1, 64.2\% variation), beak and cranial flexion (PC2, 16.7\% variation) and facial width (PC3, 5.4\% variation), together accounting for 84.6\% of total shape variation (Fig. 3). The matrix correlation between feral and domestic shape data was significant ($r_m = 0.549$, $P < 0.0001$), suggesting a similar covariance structure. This result was further confirmed by a significant non-random alignment between PC1–4 eigenvectors ($P < 0.001$) (Supplementary Table 4). Feral and domestic pigeons overlap in shapespace, although Procrustes configurations of domestics account for a larger proportion of landmark variance compared with ferals (Levene’s test, $P < 0.001$) (Supplementary Fig. 4). Domestic pigeon faces exhibited approximately eightfold higher landmark variance compared with ferals, while braincase configurations were approximately fourfold more variable. Partial least squares (PLS) analysis between face and braincase shape indicated that almost all their covariation can be explained in the first axis (94.4\%, $P < 0.001$) (Supplementary Table 5), which describes a narrowing of the braincase with increasing facial length (Fig. 4).

Finally, we compared pigeon shape data to the avian radiation. Here, brain–shape allometry accounted for 10.0\% of avian shape variation and was weakly non-significant ($P = 0.091$) (Supplementary Fig. 3). Allometric variation was associated with curvature of the beak and skull base but not with beak length, in contrast to pigeons. PCA of the non-allometric avian data decomposed cranial shape into premaxilla length (PC1, 44.4\%), skull flexion (PC2, 27.4\%) and facial width (PC3: 7.5\%) (Fig. 5 and Supplementary Fig. 5). There was evidence of significant phylogenetic signal within this shapespace ($K = 0.895$, $P = 0.042$) (Supplementary Fig. 6), with feral pigeons most closely aligned with the fruit dove and domestic breeds distributed in a space overlapping ferals, the fruit dove and the short-beaked parakeet. The avian shapespace exhibited a strong correspondence to the pigeon shape covariance structure as a whole ($r_m = 0.851$, $P < 0.001$) and individually to ferals ($r_m = 0.510$, $P < 0.001$) and domestics ($r_m = 0.777$, $P < 0.001$). Pigeon and avian shapespaces exhibited non-random alignment (angle ($\theta$) and vector correlation ($r_v$) between individual principal components: PC1: $25.2^\circ$, $r_v = 0.91$, $P < 0.0001$; PC2: 55.9°, $r_v = 0.56$, $P < 0.001$; PC3: 56.7°, $r_v = 0.55$, $P < 0.0001$; Supplementary Table 4). Avians exhibited higher disparity (−1.5x) and landmark variance (−3x) compared with domestic pigeons (Supplementary Fig. 4). RV and covariance ratio (CR) coefficients for hypothesized face–braincase modules in avians (RV = 0.76, $P = 0.098$; CR = 0.939, $P = 0.005$; r-PLS = 0.924, $P = 0.001$), ferals (RV = 0.46, $P < 0.001$; CR = 0.868, $P = 0.010$; r-PLS = 0.845, $P = 0.030$), and domestic pigeons (RV = 0.91, $P = 0.006$; CR = 1.029, $P = 0.010$; r-PLS = 0.978, $P = 0.001$) were all significant, suggesting strong shape integration (Fig. 4 and

**Figure 1** Variation in the pigeon craniofacial skeleton compared with avian diversity. **a**, Original examples of domesticated pigeon breed diversity from Darwin. **b**, Left images: modern examples illustrate notable breed variants examined in this study (see Supplementary Fig. 1 for additional examples and Supplementary Table 6 for a complete list of breeds used). Right images: examples of pigeon breeds with extreme craniofacial shapes qualitatively converge on distantly related avians. **c**, Examples of avian crania with shape characteristics similar to domesticated pigeon breeds (individual crania shown scaled to similar braincase length).
undergone little change during avian evolutionary diversification. avian craniofacial diversity is a product of selection on primitive pat beak shapes compared with brains. Together, these results imply that are accompanied by predictable but relatively small corresponding variation associated with length, curvature and width. Although face and braincase, resulting in allometric and integrated non-allometric determinants of covariation that influence evolutionary diversifica domes of birds should be similar to those of human breeders. This result shows. 

Discussion

These results demonstrate that under a regime of strong artificial selection, domestic pigeons recapitulate the principal axes of avian craniofacial shape variation, but not the magnitude. Importantly, domestic pigeons have increased the relative contributions of the same determinants of covariation that influence evolutionary diversification among avians, even though there is no reason to predict a priori that the direction of selection on functional demands in different species of birds should be similar to those of human breeders. This result indicates that both pigeon breeds and avians have diversified utilizing a common pattern of integration and modularity between the face and braincase, resulting in allometric and integrated non-allometric variation associated with length, curvature and width. Although face and braincase shape are strongly integrated, changes in facial shape are accompanied by predictable but relatively small corresponding changes in braincase shape, which accounts for the greater diversity of beak shapes compared with brains. Together, these results imply that avian craniofacial diversity is a product of selection on primitive patterns of variation and covariation between the face and braincase that are defined by a developmental system common to all birds and have undergone little change during avian evolutionary diversification.

This analysis samples a broad diversity of living birds, with species distributed across shapespace. That said, examination of predicted shapes from relatively unoccupied regions suggests that some potential outcomes may be less likely, due to a mixture of developmental and functional limitations. For example, the ‘short-face’ pigeon breeds have highly reduced premaxilla or beak size (see Supplementary Fig. 1) and are often incapable of feeding their hatchlings, requiring human intervention or surrogate parents to reach maturity. Similarly, the generation of short and curved beaks in domesticated breeds like the African owl creates functional limitations to jaw closure, which would help explain why birds with the greatest cranial or beak curvature more frequently have longer beaks. At the other extreme, avians with long beaks and unflexed crania are unsampled. This result may reflect the integrated effects of a longer braincase and narrower frontal bones, suggesting either a requirement of additional neck musculature as a balancing mechanism or a limitation on how narrow the frontal may become before affecting structural integrity. Given the relatively small changes of braincase shape compared with the face, this combination may be impacted by functional variation available in the braincase to match facial length.

The phylogenetic reconstructions within the shapespace represent testable hypotheses about both ancestral states and the developmental transformations underlying evolutionary trajectories between ancestral and descendant taxa. In the case of ancestral states, the predictions of phylogenetic hypotheses are directly testable with the inclusion of plausible ancestral taxa from the fossil record into the morphospace. Considering the developmental transformations, the trajectories are plausibly linked to previously documented developmental determinants of beak shape in avians, providing potential experimental routes for testing mechanistic explanations for interspecific variation. For example, regulation of Bmp4 expression during upper beak morphogenesis of phenotypically diverse species of the genus Geospiza impacts depth and width, while the expression of calmodulin impacts length. Variation in beak curvature may be responsive to differential regulation of local growth zones in the frontal as mass by Bmp4; alternatively, it may be a product of...
of how the inferior growth of the frontonasal mass interacts with the anterior extension of the maxillaries during primary palatal fusion. These determinants are also thought to exhibit modularity, in that they have independent effects on beak shape, consistent with our results. Future studies could directly test these hypotheses by experimentally modulating the underlying developmental determinants associated with each axis in model species and comparing the phenotypic outcomes to the predictions of the avian shapespace.

The results support the hypothesis that a conserved developmental system is sufficient to explain the majority of avian craniofacial diversification. A recent analysis reached a similar conclusion in raptors, arguing that most observed variation in this group was due to allometric scaling or non-allometric integration between the braincase and face; this suggested that developmental constraints have played an important role in the evolution of the raptor skull. There was further speculation that increasing modularity of the passerine skull might have enabled a greater relative diversification in songbirds. Although changes in clade-specific modularity may help explain relative differences in diversification, our results suggest that they are not necessary to explain diversification at the scale of all avians. Although the avian cranium is highly integrated, the magnitudes of correlated changes we identified in the braincase are small relative to those in the face (~1:3). Thus, although integration of the face and braincase influences craniofacial diversification as a whole, it does not appear to be a strict limit on the ability of the face to vary or evolve. Increased phylogenetic coverage of population-level variation of individual clades that vary widely in diversity would help to answer this question.

There are notable parallels between the patterns of variation and covariation described here and those described in the mammalian literature that suggest they are widely shared among amniotes. For example, analyses of craniofacial shape in domesticated dog breeds, wolves and carnivores found that the canine skull not only exhibits a similar pattern of integration and modularity between the face and brain, but also varies primarily in facial length, the positioning of the face relative to braincase (that is, cranial flexion) –0.10 –0.05 0.05 0.10 0.15 –0.10 –0.05 0.05 0.10 0.15 PC1 PC2 PC3 LFCL BLF GBH Italian owl Blenderine American show racer; ASR, American show racer; BLF, Berlin long-faced tumbler; BP, Brunner pouter; BSF, Budapest short-faced; DSF, Domestic show flight; ESF, English short-faced; GBH, German beauty hanner; BR, Birmingham roller; HS, Hamburg sticken; LFCL, Long-face clear leg; NYF, New York flight; PS, Polish srebrniak; VMF, Vienna medium face; WET, West of England tumbler.
and the width of the skull. A similar distribution of shape variation was observed in human craniofacial datasets. More broadly, a comparative analysis of genetic and phenotypic covariation in the skull of 15 mammalian orders found that "both remained remarkably stable for a long evolutionary period, despite extensive morphological change and environmental shifts." This stability in the pattern of variation and covariation between avians and mammals suggests that amniote craniofacial diversity varies and evolves within a shared space utilizing similar mechanisms and under similar constraints.

Why do amniote crania share a common pattern of variation and covariation? Covariation of traits within species occurs because of the correlated effects of variation in developmental processes and may result from directional selection operating on coordinated functional variation among traits. One possibility is that the embryonic bauplan of the head limits how structural components can be organized and vary relative to each other, while maintaining functional relationships. In particular, the amniote brain is derived from the neural tube, while the face has its origin in prominences arranged around the developing brain (that is, the frontonasal from nasal placodes and the maxillary from the first branchial arch, respectively), which grow together and fuse to form a continuous upper jaw. These relationships suggest the brain acts as a 'platform' on which the face grows, with both structures influencing the other's development through molecular signals, structural interactions and later somatic growth. Consequently, the width of the face is both dependent on brain size and is limited in where it is located (for example, higher or lower), impacting overall curvature or flexion of the cranium. The increased integration of brain and face variation among the domestic pigeon breeds supports this argument. Similar integration of these features across amniotes therefore suggests a fundamental limitation on the generation of functional variation imposed by the structural design of the face–braincase complex, presumably primitive in the earliest vertebrates. Similar analyses of species with extreme craniofacial shapes that violate these rules may help to determine when and how these patterns are generated.

Darwin highlighted the ability of humans to select, in a short span of time, pigeon breeds that mimicked or exceeded variation observed in wild doves. When considering the role of selection in domestication, he argued that "in the construction of a building, mere stones or bricks are of little avail without the builder's art, so, in the production of new [breeds], selection has been the presiding power." What Darwin could not appreciate at the time was that although breeders have wide latitude to generate variation, these variants mirror patterns of avian diversity at large. In the case of the avian cranium, and perhaps across vertebrates, we speculate that it is the embryonic architecture of the brain and face that shapes the space in which phenotypic diversity is possible, ultimately limiting the axes of functional variation that can be generated.

**Methods**

**Data.** To compare feral and domestic pigeons to proxies of the wildtype ancestral populations, we first obtained raw two-dimensional linear distance data for the wild rock dove (*Columba livia*, Sardinia locality; *n* = 35), feral pigeons (combined North American and European populations; *n* = 91) and domesticated pigeon breeds (*n* = 53) (see Supplementary Table 1). We next collected a sample of both feral (*n* = 15) and domestic (*n* = 58) pigeon skulls (see Supplementary Table 6), which were defleshed using dermestid beetles then scanned at a resolution of 40 μm using micro-computed tomography (μCT) (Scanco Viva40). μCT scans were thresholded and converted into three-dimensional objects in Amira 5.6.0 software (Visage Imaging). Ferals were collected at a single locality (Arlington, Texas) and domesticates were from breeders located in both California and Texas. Domestic pigeons included the following breeds (*n* = 36): Aachen langer, African owl, American fantail, American show racer, archangel, Berlin long-faced tumbler, Birmingham ‘wholly’ roller, blondinette, brunner pouter, Budapest short-faced, Chinese owl, damascene, dewlap, domestic show flight, Egyptian Baghdad, English carrier, English short-faced, German beauty homer, Hamburg sticken, helmet, Indian fantail, Italian owl, Lahore, long-face clear leg, magpie, Maltese, Moderna, mooskee, New York flight, Polish ice tumbler, Polish srebrniak, roller, scandaroon, swallow, Vienna medium face and West of England tumbler.
To compare pigeon shape to the larger avian radiation, we next sampled avian crania (n = 20) representing 17 orders and encompassing a range of beak shapes, sizes and functions (Supplementary Table 6): black-naped fruit dove (Ptilinopus melanopilus, Order: Columbiformes), kiwi ( Apteryx australis, Order: Tinamiformes), ostrich (Struthio camelus, Order: Struthioniformes), chicken (Gallus gallus, Order: Galliformes), white pekin duck (Anas platyrhynchos, Order: Anseriformes), black vulture (Coragyps atratus, Order: Accipitriformes), little pigeon (Euleptula minor, Order: Sphenisciformes), emperor penguin (Aptenodytes forsteri, Order: Sphenisciformes), Brandt’s cormorant (Phalacrocorax penicillatus, Order: Pelecaniformes), frigatebird (Fregata magnificens, Order: Pelecaniformes), common loon (Gavia immer, Order: Gaviiformes), giant hummingbird (Patagia gigas, Order: Apodiformes), black vulture (Coragyps atratus, Order: Accipitriformes), little pigeon (Euleptula minor, Order: Sphenisciformes), emperor penguin (Aptenodytes forsteri, Order: Sphenisciformes), Brandt’s cormorant (Phalacrocorax penicillatus, Order: Pelecaniformes), frigatebird (Fregata magnificens, Order: Pelecaniformes), common loon (Gavia immer, Order: Gaviiformes), giant hummingbird (Patagia gigas, Order: Apodiformes), white-tipped sedgebill (Eutoxeres australis, Order: Anseriformes), golden-winged parakeet (Brotogeris chrysoptera, Order: Psittaciformes), grebe (Podiceps nigricollis, Order: Podicipediformes), albatross (Diomedea exulans, Order: Procellariiformes), auk (Alca torda, Order: Charadriiformes), red-tailed tropicbird (Phaethon rubricauda, Order: Pteroclidiformes) and manakin (Lepidothrix coronata, Order: Passeriformes). Avian computed tomography (CT) data were obtained courtesy of the University of Texas High Resolution X-ray CT Facility (UTCT; www.DigiMorph.org).

Linear analysis. We first performed a reduced major axis linear regression of cranial measurements on size (estimated as the geometric mean from both cranial and postcranial measurements). We tested for significant differences in elevation in the smatr module16, implemented in the software R (ref. 17). We next compared trait correlation structure via the matrix correlation (r_m), adjusted for repeatability (that is, r_{m,adj} = (r_{m,corr} / (1 - r_{E}^2))^{1/2}), where r is the matrix repeatability of matrices a and b) and resampled each population with replacement (10,000 replicates) to estimate autocorrelation, before testing for significance using Mantel’s test18. To estimate and compare the magnitude of integration we calculated the variance of the eigenvalues (v.e.) for each correlation matrix18,20. To control for the effect of sampled variance on correlation and eigenvalue estimates, we followed ref. 18 and resampled each population with replacement and recalculated v.e. and the average c.v. of all cranial measurements for each replicate (10,000). We next calculated the log-linear relationship of c.v. to v.e. in those samples where r_m > 0.95 with the original matrix and compared populations at similar c.v. values.

Shape analysis. We next used geometric morphometric methods to quantify and compare shape variation and covariation among feral and domestic pigeons and the avian radiation. For each cranium, we identified a series of 32 midline and bilaterally symmetric three-dimensional landmarks evenly divided between the face and braincase (see Supplementary Fig. 9 and Supplementary Table 7).
Individual specimen coordinates (x, y, z) were located in Landmark Editor (v.3.5; http://graphics.idv.anadis.edu/research/EvolveMorph) using a semi-automated process that was repeated on two separate occasions. We compared between landmarking trials and noted low error, so we averaged coordinates between trials. We next performed a Procrustes superimposition on all the landmark data to remove the effects of scale, translation/location and orientation. Data were averaged across the plane of bilateral symmetry to reduce dimensinality relative to sample size. We also averaged Procrustes data and centroid sizes within each breed and/or species to account for unequal sample sizes.

To test and control for significant size–shape allometry, we first calculated disparity and landmark variance for the entire craniofacial skeleton as well as within the braincase and face modules. We noted that facial shape variation was significantly increased whereas braincase shape was more conservative (Supplementary Fig. 10). We also collected published measures of body mass and brain volume for all species in our analysis (see Supplementary Table 6).

As previously demonstrated, brain–body allometry is significant in avians (\( r^2 = 0.888, P < 0.0001 \) (Supplementary Fig. 10), indicating brain volume is a good proxy for body mass. For the pigeon data, information on individual body mass was not collected, so we instead used braincase centroid size as the measure of size. For comparisons among avians, we used log(brain volume) as within cranio proxy to remain consistent with the pigeon analyses and to test for size–shape allometry in our cranial data. We performed a within-group multivariate regression of shape on braincase centroid size in pigeons (feral and domestic; see ‘Data’ above). To compare between pigeon and avian brain–shape allometry, we centred average pigeon log(brain volume) on the mean feral pigeon braincase centroid size.

To compare the distribution of shape variation and covariation in the non-allometric shape data, we performed PCA on residuals of brain–shape allometry. To compare overall covariation structure similarity we calculated \( r_p \) and tested the hypothesis of no similarity using a permutation test (10,000 replicates). We further compared covariance structure by calculating the angle (\( \phi \)) and vector correlation (\( r_v \)) between individual principal components and tested whether they were significantly better aligned than predicted by chance alone using a permutation test (1,000 iterations). To investigate integration and modularity of the face and braincase in the individual shape datasets, we performed a PLS test and modularity test functions in the R package geomorph (ref. 41). As previously demonstrated, the shapes of bird brains are highly controlled by nondietary factors. Proc. Natl Acad. Sci. USA 113, 5352–5357 (2016).

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Author contributions
N.M.Y. designed the research. N.M.Y. and J.W.F. collected pigeon specimens. N.M.Y. and M.L.-M. performed the analyses. N.M.Y., M.L.-M., B.H., and R.S.M. contributed to the interpretation of the results. N.M.Y. drafted the paper. All authors contributed to the final version of the paper.

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Competing interests
The authors declare no competing financial interests.