Bringing Up Baby: Developmental Simulation of the Adult Cranial Morphology of *Rungwecebus kipunji*

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ABSTRACT

*Rungwecebus kipunji* is a recently discovered, critically endangered primate endemic to southern Tanzania. Although phenetically similar to mangabeys, molecular analyses suggest it is more closely related to *Papio* or possibly descended from an ancient population of baboon-mangabey hybrids. At present, only a single kipunji specimen, an M1-stage juvenile male, is available for study; thus, the cranial morphology of the adult kipunji is unknown. In this study, we used developmental simulation to estimate the adult kipunji's 3D cranial morphology. We examined variation in cercopithecine developmental vectors, applied selected vectors to the juvenile cranium, and compared the resulting simulated adults to actual adult male papionins. Differences between papionin developmental vectors were small and statistically insignificant. This uniformity suggests conservation of an ancestral papionin developmental program. Simulated kipunji adults were likewise extremely similar. As a group, the simulated adults were morphometrically distinct from other papionins, corroborating the kipunji's generic status. Simulated adults were phenetically most similar to *Lophocebus aterrimus* but were distinguished from all adult papionins by the same unique traits that characterize the kipunji juvenile: a tall neurocranium, broad face, short nasal bones, concave antorbital profile, and dorsally rotated palate. This concordance between juvenile and estimated-adult morphologies confirms that papionin cranial shape is largely established before M1 eruption. The estimated kipunji adult's neurocranium strongly resembles that of *Papio*, providing the first cranial evidence supporting their phylogenetic relationship. If the kipunji does indeed have a hybrid origin, then its phenetic affinity to *L. aterrimus* favors *Lophocebus* as the proto-kipunji's paternal lineage. Anat Rec, 293:388–401, 2010. © 2009 Wiley-Liss, Inc.

Keywords: mangabey; *Lophocebus*; *Papio*; hybrid origin; permutation test

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INTRODUCTION

The kipunji, *Rungwecebus kipunji* (Cercopithecinae: Papionini), is a critically endangered primate endemic to the Udzungwa Mountains and Southern Highlands of Tanzania (Rovero et al., 2009). Following its discovery in 2003, the kipunji was referred to the mangabey genus *Lophocebus* (Jones et al., 2005; Ehardt and Butynski, 2006), but subsequent molecular phylogenetic analyses found it to be the sister taxon to the baboon genus *Papio* (Davenport et al., 2006; Olson et al., 2008). To accommodate this new papionin, the monotypic genus *Rungwecebus* was created (Davenport et al., 2008). Most recently, further mitochondrial studies (Burrell et al., 2009; Zinner et al., 2009) have found evidence that the kipunji originated through hybridization between female yellow baboons (*Papio hamadryas cynocephalus*) and as yet unidentified, mangabey-like sires.

The genetic material used in these studies derives from a single, juvenile male voucher specimen. This specimen's immaturity prevents the evaluation of many characters considered diagnostic for African papionins (Fleagle and McGraw, 2002; Davenport et al., 2006; McGraw and Fleagle, 2006; Gilbert, 2007), hindering conventional assessments of its morphological affinities. Singleton's (2009) comparative analysis of subadult papionin cranial shape found the kipunji juvenile to be morphometrically distinct from other papionins at a level consistent with generic status and to have stronger phenetic affinities to macaques and *Cercocebus* than to its closest relatives, *Lophocebus* and *Papio*.

Singleton's findings raise at least as many questions as they answer, foremost among them the nature of adult kipunji morphology. Although several studies have shown that taxonomic differences in primate cranial shape are established early in development (O'Higgins and Collard, 2002; McNulty et al., 2006; Singleton, 2009), it has also been demonstrated that convergence of ontogenetic trajectories can diminish or eliminate juvenile shape differences between papionin species (Leigh, 2007). If shape differences cannot be assumed to be stable throughout ontogeny, alternate methods must be used to infer the adult morphology of isolated juvenile specimens. Morphometric simulation can be a powerful tool for evaluating taxonomic affinities of unknown specimens (Richtsmeier and Walker, 1993; McNulty et al., 2006; Baab and McNulty, 2008). We adopted this approach here and estimated adult kipunji cranial morphology by applying a range of cercopitheine developmental vectors to the original kipunji specimen. Simulated adults were compared with adult males of other papionin species and to each other to evaluate the effect of developmental model choice, characterize adult kipunji cranial morphology, and explore the kipunji adult's phenetic affinities.

BACKGROUND

Papionin Phylogeny and *Rungwecebus* Systematics

The Old World monkey tribe Papionini (Primates: Cercopithecinae) encompasses two geographically segregated subtribes (see Fig. 1). The predominantly Asian macaque radiation (*Macaca*) is the sister taxon to the predominantly African Papionina, which encompasses mangabeys (*Cercocebus, Lophocebus*), mandrills (*Mandrillus*), baboons (*Papio*), and geladas (*Theropithecus*) (Szalay and Delson, 1979; Strasser and Delson, 1987). Molecular phylogenetic analyses have consistently shown the mangabeys to be diphyletic; *Cercocebus* and *Mandrillus* constitute a sister clade to the *Lophocebus-Papio-Theropithecus* (LPT) clade, whose internal phylogenetic relationships remain unresolved (Barnicot and Hewett-Emmett, 1972; Cronin and Sarich, 1976; Hewett-Emmett et al., 1976; Disotell, 1994; Van Der Kuyl et al., 1995; Harris and Disotell, 1998; Page et al., 1999; Harris, 2000).

Though it no longer references a monophyletic group, the term “mangabey” has persisted as convenient shorthand for ecomorphologically similar, medium-sized African papionins characterized by moderate facial prognathism, well-developed suborbital fossae, hard-object diets, bunodont molars, and varying degrees of arboreality (Kingdon, 1997). The mangabey phenomenon contrasts with the large-bodied, long-faced, predominantly terrestrial “baboons,” *Mandrillus, Papio*, and *Theropithecus*. The extensive homoplasy implied by the phylogenetic distribution of these ecomorphs has made African papionins a touchstone for the study of primate morphological variation and evolution (Lockwood and Fleagle, 1999; Collard and O’Higgins, 2001; Singleton, 2002; Leigh, 2007).

From its initial description in 2005, the kipunji was identified as a mangabey. Jones et al. (2005), citing the kipunji’s noncontrasting eyelids and arboreal habitus, referred it to *Lophocebus* as a new species, *L. kipunji*, but noted differences from Central African *Lophocebus* species in pelage, tail carriage, and vocalization. This matter was revisited when the first physical voucher specimen, an M1-stage juvenile male with permanent incisors erupting (see Fig. 2), provided genetic material for phylogenetic analysis (Davenport et al., 2006; Olson et al., 2008). Mitochondrial sequences (12s rRNA, COI, and COII) consistently linked the kipunji with *Papio* to
the exclusion of Lophocebus. Results based on nuclear sequences were mixed, with two sequences (X-chromosome intergenic region, LPA) strongly supporting the Papio-kipunji sister relationship, one (1, 3-GT) providing weak support, and two (TSFY, CD4) failing to resolve the LPT clade. These results, as well as the apparent absence of shared features between the kipunji and Papio, led Davenport et al. (2006) to erect a new genus, Rungwecebus.

More recently, two independent studies (Burrell et al., 2009; Zinner et al., 2009) have shown that kipunji mitochondrial sequences (COI, COII) nest within the Papio clade (see Fig. 1). Specifically, its haplotypes group with southern populations of yellow baboons (Papio hamadryas cynocephalus) from the geographic vicinity (~325 km) of Mt. Rungwe. In these studies, divergence estimates for the kipunji and P. h. cynocephalus mitochondrial lineages range from 0.65 Ma (95% CI: 1.34–0.25 Ma; Burrell et al., 2009) to 0.35 Ma (95% CI: 0.67–0.09 Ma; Zinner et al., 2009). Both research groups interpret these findings as evidence of ancient hybridization between yellow baboon females and, based on the kipunji’s phenotype, males of an unidentified mangabey species. Burrell et al. (2009) favor Lophocebus as the source of the paternal population but, on the basis of reported phenetic similarities (Singleton, 2009), do not rule out Cercrocebus. Zinner et al. (2009) postulate a Papio-Rungwecebus divergence (i.e., a true sister-group relationship) followed by introgressive hybridization between an isolated southeastern yellow baboon population and proto-kipunji males. In both scenarios, the modern kipunji is hypothesized to have descended from a population of reproductively viable, intergeneric hybrids.

Phylogenetic relationships within the LPT clade are unresolved due primarily to the extremely short (estimated < 400,000 years) divergence intervals (internodes) between its constituent taxa (Disotell, 1994; Harris and Disotell, 1998; Harris, 2000, 2002). This uncertainty, coupled with the limitations of the available kipunji sequences, makes resolution of the kipunji’s nuclear-DNA relationships problematic; thus, morphological corroboration of the molecular results is extremely desirable. Davenport et al. (2006) described the kipunji juvenile cranium as possessing classic Lophocebus features such as relatively narrow zygomatic breadth, zygo-

Developmental Simulation

The dilemma posed by the kipunji juvenile is analogous to that encountered by paleontologists, who must infer the adult morphology of juvenile fossils to determine their taxonomic affinities (e.g., Tobias, 1978; Alemseged et al., 2006; McNulty et al., 2006). In such cases, developmental simulation of adult cranial shape has proved useful for exploring ontogenetic differences among species and for testing taxonomic hypotheses (Richtsmeier et al., 1993; Richtsmeier and Walker, 1993; Ackermann and Krovitz, 2002; McNulty et al., 2006). McNulty et al.’s (2006) simulation of the Taung child’s adult facial morphology using extant great ape
developmental models is the most recent exemplar of this approach. They found that even when developmental trajectories are significantly different, adults created using extant juveniles of one species and the developmental trajectory of a different species consistently classified to the original juvenile’s species (McNulty et al., 2006). These findings demonstrate the robustness of developmental simulation as a method of examining species-level morphological differences.

In this study, we used a similar developmental simulation to infer the adult cranial morphology of *Rungwecebus*. To select appropriate models, we first examined differences among male cercopithecine developmental patterns and then simulated adult kipunji cranial shape using a range of these models. The resulting simulated kipunji adults were compared with adult male papionins and with each other to: (1) explore variation in papionin developmental patterns; (2) assess the effect of developmental model choice; (3) characterize adult kipunji cranial morphology; and, (4) evaluate the adult kipunji’s phenetic affinities.

**MATERIALS AND METHODS**

**Materials**

The study sample comprised 205 cercopithecine crania representing all papionin genera and two cercopithecine outgroups (see Table 1). The majority were wild-shot specimens of known provenience, but zoo specimens judged to present normal (i.e., nonpathological) morphologies were included for taxa (e.g., *Mandrillus*, *Theropithecus*) otherwise poorly represented in museum collections. Criteria for specimen selection included cranial symmetry, absence of dental anomalies, and normal bone texture and density. Specimens were drawn from the collections of the Royal Museum for Central Africa (Ter- vuren), the Royal Belgian Institute of Natural Sciences (Brussels), the Tulane University Museum of Natural History (New Orleans), the American Museum of Natural History (New York), and the Field Museum of Natural History (Chicago).

Specimens were assigned to developmental categories on the basis of dental eruption. Dental stages were defined by eruption of the nominal tooth to full occlusion. Thus, in an M1-stage individual, M1s are fully erupted and wearing but M2s are not yet in occlusion. Because subadult specimens are scarce and their sexes frequently unknown, use of mixed-sex samples was necessary to obtain reasonable juvenile sample sizes. This is justified by prior findings that male and female ontogenetic trajectories do not typically diverge until late in ontogeny (O’Higgins and Jones, 1998; O’Higgins and Collard, 2002; Leigh, 2006). As the only known kipunji specimen is a male, this study focused on male developmental patterns and adult females were not sampled.

**Data Collection and Processing**

Three-dimensional craniometric landmarks and cranial contours were collected and processed following a previously published protocol (Singleton, 2009). Contours were reduced to equal numbers of equidistant semi-landmarks using *Resample* (Raaum, 2006) and treated as standard landmarks during superimposition. The final landmark configurations, comprising 112 landmarks and 41 semi-landmarks (see Singleton, 2009), were aligned using generalized Procrustes analysis (GPA) in *Morph* (Slice, 1998). This procedure removes the effects of translation, rotation, and scale, thus bringing all specimens into a common shape space (Gower, 1975; Rohlf and Slice, 1990; Dryden and Mardia, 1998). Following superimposition, missing landmarks were estimated either by reflection of antimeres or by substitution of stage-specific species-mean coordinates (see Slice, 1996; Singleton, 2002). Reflection and estimation were executed using SAS 9.1 IML modules (SAS Institute, Cary, NC). Species adult means were computed for males only; subadult dental-stage means were computed using pooled-sex samples, which provide reasonable estimates for subadults of both sexes (Singleton, 2009). Complete configurations were then subjected to a second GPA alignment. As Procrustes-aligned coordinates have nearly perfect correspondence with their Euclidean tangent-space projections (Marcus et al., 2000; McNulty et al., 2006; Singleton, 2009), aligned coordinates were used in all analyses.

**Calculation and Comparison of Developmental Vectors**

The developmental trajectory for each papionin species was approximated by linear regression of Procrustes-aligned coordinates, that is, cranial shape, on a four-state categorical (dummy) variable representing dental
Developmental Simulation

Cercopithecine developmental trajectories were applied to the juvenile kipunji cranial landmark configuration to create a series of estimated kipunji adults. Juvenile samples of *Mandrillus leucophaeus* and *Theropithecus gelada* were either unavailable or too sparse to provide reliable simulations; however, adult males of these species were included in subsequent comparative analyses. To model the kipunji’s transformation from M1-stage juvenile to adult male, developmental vectors of the remaining species were recalculated excluding dp4 juveniles. Just as bivariate regression coefficients express change in a dependent variable per unit change of the independent variable, the multivariate developmental vector summarizes cranial shape transformation at each landmark coordinate between successive dental stages. To represent shape change from M1 eruption (stage 2) to adulthood (stage 4), the developmental coefficient vector is multiplied by a factor of two, the number of stage changes. Addition of this augmented vector to the landmark coordinates of the kipunji juvenile cranium represents its simulated adult landmark configuration (McNulty et al., 2006).

Visualization of Simulated Adults

To aid in the visualization of the simulations, an anatomically accurate surface model of the juvenile kipunji cranium, FMNH 187122, was created using a NextEngine desktop 3D laser scanner. Multiple scan views were aligned and merged in Rapidform® XOR (Rapidform, Inc.) to produce a single, complete model. Estimates of adult kipunji cranial shape were visualized by applying the morphing function of *Landmark* 3.0.0.6 (Wiley et al., 2005) to this model. For visualization purposes, symmetrical landmark configurations were created for the juvenile and simulated kipunji adults (SKAs). Each landmark configuration was mirror imaged by exchanging the coordinates of its right and left landmarks after first adding missing-data values as placeholders for the nonexistent left semi-landmarks. Mirror images were superimposed in *Morphus* and averaged to yield a single, symmetrical configuration with semi-landmarks on both right and left sides. The surface model and symmetrical landmark configurations were then loaded into *Morpheus*, and the juvenile kipunji landmarks were transferred to the model surface. Three-dimensional representations of the adult kipunji cranium were created by morphing the juvenile model to each adult landmark configuration, using corresponding landmarks to direct the transformation and thin-plate splines to interpolate the shape transformations between landmarks (Bookstein, 1991; Wiley et al., 2005). Comparisons were made among SKAs to examine the influence of different developmental vectors on the estimated adult shape.

Shape differences between SKAs and actual adults of other species were also explored graphically by superimposition of symmetric cranial landmark configurations in *Morphus* (Slice, 1998). Although less detailed than the surface visualizations, these wireframe representations facilitate the identification of regional shape differences between superimposed configurations. Comparisons of the average adult kipunji shape to mean adult-male shapes of other papionins were used to assess the kipunji’s phenetic resemblance to other species.

Morphometric Affinities of Simulated Adults

A principal components analysis (PCA) of actual papionin adults and SKAs was used to locate simulated adults relative to other species and to each other in a reduced-dimension cranial morphospace. Quantitative comparisons of cranial shape differences between SKAs and adult males of other species were based on the Procrustes distance, which is equivalent to the Euclidean distance in shape space between two optimally superimposed specimens (Bookstein, 1991). Procrustes distances were calculated between each actual adult specimen and its species mean, and descriptive statistics were calculated from these to determine the level of shape variation expected within species. Procrustes distances between SKAs and the average simulated adult were compared with these species distributions to assess the consistency of the adult estimates and the effects of different developmental models on the estimated adult morphology.

The phenetic affinities of individual SKAs were tested using three different Procrustes distance-based criteria. First, we determined affinity according to the nearest
Comparison of Developmental Vectors

Table 2 shows pairwise angular differences between species developmental vectors. The gelada vector was most dissimilar, with interspecific angles in the range of 41–47°, followed closely by Mandrillus sphinx (37–44°) and the Kinda baboon (24–41°). Angular differences between developmental vectors of small-bodied papionins ranged from 14° to 29°. None of these differences were significant, however, and even the Theropithecus gelada angles are relatively small given the large number of landmarks used in this study. The relative uniformity of papionin developmental trajectories, irrespective of phylogenetic affinity, is apparent in the dendrogram of angular differences (Fig. 3a). The pairing of the cercopithecins, Allenopithecus nigroviridis and Chlorocebus aethiops, is the only indication of phylogenetic signal, whereas the papionin vectors (Mandrillus and Theropithecus excluded) comprise an unstructured cluster.

Morphometric Affinities of Simulated Adults

The uniformity of papionin developmental vectors is also reflected in the morphometric similarity of the simulated kipunji adults derived from them. The phenotype of simulated and actual papionin adults clusters the majority of the SKAs to the exclusion of other small-bodied cercopithecines (Fig. 3b). Within the SKA cluster, the pairings of the A. nigroviridis and Ch. aethiops simulations and the C. torquatus simulation and C. torquatus, respectively, show some influence of taxonomic vector differences on estimated adult morphologies, but taxonomic patterning is otherwise absent. Only the Mandrillus sphinx simulation shows a strong, exclusive affinity to its developmental species.

Similarity among SKAs is confirmed by comparisons of Procrustes distances between individual SKAs and the mean simulation (Table 3), which reflect a level of shape variation similar to that found within papionin species. These results indicate that different developmental models produce estimates of the adult kipunji morphology that are no more different than members of a single species. The exception is the simulation generated from the vector of M. sphinx, whose larger Procrustes distance (see footnote to Table 3) rules it out as a reasonable estimate of the adult kipunji morphology.

On principal component plots of actual and simulated adults (Fig. 4a), most SKAs cluster tightly, implying that our estimates of adult morphology are precise, and do not overlap the distributions of other papionins. Simulations based upon the Chlorocebus, Papio, and Mandrillus vectors fall away from the core group in the directions of their respective model taxa. Only the latter is substantially removed from the kipunji cluster, perhaps reflecting the extended development of male mandrills (Setchell et al., 2001; Setchell and Wickings, 2004; Setchell et al., 2006). On PC1, the M. sphinx simulation falls outside the core papionin group in the direction of mandrills and drills. On PC3, the remaining SKAs fall closest to, but well separated from, Cercocebus and Lophocebus, again suggesting that the kipunji adults are more different from these genera than either is from the other. Consistent with this finding, the average SKA clusters as the sister group to other small-bodied papionins (see Fig. 4b) suggesting that it is phenetically distinct from other mangabeys and from macaques.

Procrustes distances between the SKAs and other adult papionins indicate a strong affinity between R. kipunji and Lophocebus aterrimus but also confirm that the kipunji is a distinct phenon. Under the nearest neighbor criterion (see supplemental online material Table S1), all SKAs but one are closest to L. aterrimus; the C. agilis-based simulation (K_Cga) falls closest to its vector species, C. agilis. When sorted according to the nearest species mean (SOM, Table S2), all but three SKAs are closest to L. aterrimus. The K_Ctt and K_Msp simulations fall closest to C. torquatus, whereas K_Cta falls nearest to C. atys. Comparisons based on nondiscrete developmental simulations (SOM, Table S3) yield essentially the same results, with every developmental vector passing closest to the mean of L. aterrimus except that of the K_Ctt simulation, which runs closer to C. torquatus. When compared with the intraspecific developmental simulations (cf. McNulty et al., 2006). Instead of simulating a discrete adult configuration, the SKAs developmental vector was assumed to be infinite and affinity was determined by the species mean with the smallest perpendicular Procrustes distance to this vector at any point along its trajectory.

RESULTS

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Procrustes-distance distributions (Table 3), none of the simulated kipunji adults falls within the adult-male range of variation of any species examined. Procrustes distances between adult-male papionins and the average estimated kipunji adult also equal or exceed distances between members of different genera (Table 4). This is further evidence that the kipunji cannot be accommodated within any papionin taxon sampled and corroborates Singleton's (2009) finding that the kipunji represents a distinct genus.

Comparisons of Estimated Adult Morphology

Consistent with the PCA and cluster results, the visualizations of the simulated adult crania are extremely similar to each other and to their average shape (Fig. 5). Where individual simulations deviate from the average, they do so in ways that are consistent with the morphology of the vector species. Thus, the Lophocebus simulations (K_Laa and K_Laj) have more anteriorly positioned zygomatic roots, deeper suborbital fossae, and lower neurocranial. The simulation based on the vector of the Kinda baboon (K_Phk) has a higher frontal bone, more prominent glabellar region, more globular neurocranium, and greater facial prognathism. The C. torquatus simulation (K_Ctt) is also relatively prognathic, with shallower suborbital fossae and somewhat inflated maxillary ridges. The SKAs’ mutual similarities, however, clearly outweigh their differences, and this consistency recommends the average SKA as a robust estimate of the kipunji’s adult cranial morphology (Fig. 5, K_Mean).

Comparisons between the average SKA and the mean adult-male shapes of actual papionins reveal cranial shape differences consistent with those described by Singleton (2009) for the juvenile cranium (Figs. 6 and 7). In comparison with most other papionins—including L. aterrimus, the taxon to which it is most similar—the adult kipunji (average SKA) exhibits a taller, broader neurocranium; broader face and suborbital fossa; more concave antorbital profile; shorter nasal bones; and a more dorsally rotated palate. In contrast with L. aterrimus, in particular, the adult kipunji exhibits broader, less restricted suborbital fossae, and its zygomatic arches are less sinusus and more laterally positioned. In contrast with the juvenile specimen (Singleton, 2009), the simulated adult neurocranial length is relatively greater than that of L. aterrimus but similar to L. albigena and, interestingly, Papio hamadryas kindae. The adult kipunji’s cranial base is most similar to that of L. aterrimus, whereas its frontal bone shape most resembles C. agilis. The kipunji’s neurocranial height and overall shape are most similar to that of P. h. kindae, whereas

**Table 3. Descriptive statistics for within-species Procrustes distances to adult-male species means**

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<th>Max.</th>
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*The simulation based on M. sphinx is excluded from these calculations because its Procrustes distance to the mean (0.1423) indicates a much greater magnitude of developmental shape change, clearly ruling it out as an appropriate model.
its facial profile is closest to that of Macaca mulatta. The short palate of the simulated adult is likely an artifact of the abbreviated premaxillary alveolar process of the juvenile specimen, whose incisors are not fully erupted.

DISCUSSION

The curious case of the kipunji is a pointed reminder that modern primatologists work in a very different milieu from that of their predecessors. Truly new primate species, much less genera, are exceedingly rare and often, like the kipunji and Arunachal macaque (Sinha et al., 2005; Chakraborty et al., 2007), are Red Listed as soon as they are described (Davenport and Jones, 2008; Kumar et al., 2008). Ethologists, morphologists, and molecular phylogeneticists must race poachers and habitat destruction to characterize and understand newly discovered primates while they still exist in their natural environments. Conservation-mandated scarcity of physical voucher specimens and the steady attrition of irreplaceable comparative collections require that bioanthropologists embrace new approaches to the study of primate morphological diversity. Increasingly, we will need to adopt minimally destructive methods.
TABLE 4. Procrustes distances among cercopithecine adult-male means

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aAverage estimated adult kipunji.
Abbreviations follow Table 1.

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Fig. 5. Three-dimensional representations of simulated kipunji adults (SKAs). SKAs based on different species vectors (periphery) are scaled to unit size to facilitate shape comparisons. The virtual juvenile cranium (center) is scaled to 85% of adult size, the approximate ratio of the juvenile cranial centroid size to the adult-male average centroid size for Lophocebus. Each SKA is identified by “K_” plus the taxonomic label (following Table 1) of the species whose vector was used to create it. K_Mean is the mean of the other simulations. Differences among the SKAs are minor, which suggests that the estimate of adult-male kipunji morphology is robust.
that maximize information gleaned from limited samples and preserve existing comparative material for future researchers, making conservation the watchword in the collection as well as in the field.

In this study, we apply one such method, developmental simulation, to the sole physical specimen of a unique and critically endangered primate, the kipunji. By simulating the kipunji's adult cranial morphology using a range of developmental models, we sought to gain a fuller understanding of the kipunji's morphology and taxonomic affinities as well as new insights into papionin cranial development. Among the questions addressed were: How different are papionin developmental patterns? How much does the choice of developmental model affect estimates of adult cranial shape? What is the contribution of pre-M1 morphogenesis to adult cranial morphology? and, How do estimated kipunji adult crania compare to those of actual papionins? Unlike similar analyses of fossil material, our results are ultimately testable once adult specimens become available.

**Developmental Simulation**

Differences between species’ developmental vectors (Table 2) were remarkably uniform across the three major papionin clades and, in all cases, insignificant. In the cases of *Mandrillus sphinx* and *Theropithecus gelada*, species with large angles but small subadult samples, lack of statistical significance may be attributable to small sample sizes. Our results contrast those of Leigh (2007), who found developmental trajectories in *Papio* to diverge strongly from those of other papionins. Leigh associated this divergence with increased body size in the *Papio* lineage, so our discrepant results may be attributable to our use of *P. h. kindae*, the smallest extant baboon. The failure of papionin vectors to cluster by genus (Fig. 3a) implies that small-bodied papionins share a common developmental pattern distinct from that of cercopithecins. That this pattern appears to be present in both *Macaca* and in African forms suggests it is retained from the common papionin ancestor.

In morphometric and visual comparisons, simulated kipunji adults (SKAs) based on different papionin vectors are extremely similar. The phenogram of actual and simulated adults (Fig. 3b) clusters simulated kipunjis to the exclusion of other small-bodied papionins. The general failure of simulated kipunji adults to cluster according to vector source further corroborates the uniformity of papionin developmental patterns. That said, the pairing of simulations based on *Cercocebus torquatus* and

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Fig. 6. Superimposition of the average adult kipunji (blue) on the adult-male averages of selected papionin species (red). In lateral view, the kipunji is distinguished from most papionins by its relatively tall neurocranium, short nasal bones, concave antorbital profile, and dor-sally rotated palate. Its neurocranial height and overall shape are most closely matched by *Papio* (Phk). The simulated adult's snubbed palate is an artifact of the kipunji juvenile's abbreviated premaxillary alveolar process. Taxon abbreviations follow Table 1.
Mandrillus sphinx with actual C. torquatus and Mandrillus adults suggests unique species-vector influences on estimated adult shape. This is particularly interesting given the recent conjecture that C. torquatus is more closely related to Mandrillus than to other species of Cercocebus (McGraw and Fleagle, 2006).

The effects of different species vectors on estimated adult shape are seen in the taxonomic clustering of these two SKAs as well as in subtle, taxon-typical deviations of individual simulations from the average estimate (Fig. 5). Angular differences between developmental vectors, some of which (e.g., angles for Mandrillus) may prove statistically significant with larger samples, certainly contribute to these deviations; however, differences in vector length—not investigated in this study—may also play an important role in establishing adult shape differences. Yet even the most divergent simulation—the adult based on the vector of Mandrillus sphinx—is as similar to other simulated kipunjis as it is to an adult male mandrill. The consistency of estimated adult cranial shapes suggests that the developmental-simulation approach employed in this study has produced a reliable estimate of adult kipunji morphology. The similarity of the cranial traits present in the original juvenile specimen and simulated adult kipunjis further corroborates studies showing that interspecific differences in primate cranial shape are well established before the M1 dental stage (O’Higgins and Collard, 2002; McNulty et al., 2006; Singleton, 2009). It appears that early juvenile morphogenesis establishes a cranial plan that subsequent development has only limited ability to modify.

**Phenetic Comparisons**

Singleton (2009) found the juvenile kipunji cranium to be phenetically most similar to juveniles of Macaca and Cercocebus but morphologically distinct from all papionins at a level consistent with generic status. In the present study, Procrustes distance-based determinations of phenetic affinity (Tables S1–S3) almost unanimously identify Lophocebus aterrimus as being most similar to the adult kipunji’s morphology. However, the SKAs’ position outside the main papionin cluster (Figs. 3b and 4b), their separation from other papionins in PCA space (Fig. 4a), and their total lack of overlap with extant species’ Procrustes-distance distributions (Tables 3 and 4) confirm that the kipunji represents a distinct morphology. Moreover, the kipunji adult’s average Procrustes distances to other mangabey species (Table 4) are comparable with distances between the two mangabey genera. By this
measure, the kipunji is as deserving of generic recognition as either Cercocebus or Lophocebus, if not more so.

In visual comparisons, the adult kipunji is distinguished from all other papionins by its relatively tall, broad neurocranium, broad face and suborbital fossae, concave anteorbital profile, and dorsally oriented palate, that is, the differences identified by Singleton (2009) as distinctive of the juvenile kipunji cranium. As in the previous study, the estimated kipunji adult shares no features with Lophocebus to the exclusion of other taxa, and it lacks certain features—such as a low cranial vault, sinuous zygomatic arch, and laterally restricted suborbital fossa (see Figs. 6 and 7)—associated with Lophocebus (Groves, 1978). Rather, the kipunji cranium exhibits a generalized, mangabey morphotype as similar to Cercocebus in many particulars as to its closer relative and consistent nearest neighbor, L. aterrimus. The kipunji’s distinctive neurocranial shape does resemble that of the Kinda baboon, the only hint, to date, of any cranial similarity between the kipunji and Papio. This resemblance is noteworthy given that Papio is characterized by accelerated perinatal brain growth and, thus, a relatively paedomorphic (for its size) neurocranial shape (Leigh, 2007). Whether the kipunji’s neurocranial height reflects a similar ontogenetic dissociation is a topic worthy of future investigation.

**Kipunji Evolutionary Relationships and Systematics**

The kipunji adult’s phenetic distance from other species, lack of unambiguous synapomorphies with other taxa, and unique cranial characteristics reaffirm that the kipunji is, by present standards, worthy of generic recognition. That said, many questions concerning the origins and evolutionary history of Rungwecebus remain unresolved.

The kipunji’s cranial morphology is a mosaic, sharing traits with Lophocebus, Cercocebus, and even Macaca, although few with Papio. To explain this distribution, Singleton (2009) proposed a scenario based on character sorting, with the kipunji retaining more primitive traits as the lineages of the LPT clade diverged. Recent evidence (Burrell et al., 2009) that kipunji mitochondrial sequences nest within extant Papio hamadryas cynocephalus (Fig. 1) has given rise to two additional scenarios: (1) the kipunji is a yellow-baboon descendant that has convergently evolved a mangabey-like morphology and habitus; (2) the kipunji arose through ancient hybridization between southeastern yellow baboon females and male mangabeys. The latter hypothesis is the more plausible, but no nuclear sequence links the kipunji to Lophocebus (or any other taxon) rather than to Papio (Burrell et al., 2009; Zinner et al., 2009). Citing the juvenile kipunji’s phenetic resemblance to Cercocebus (Singleton, 2009), Burrell et al. (2009) left open the question of this hypothetical hybrid’s paternity. Zinner et al. (2009), apparently giving greater credence to nuclear DNA phylogenies that link the kipunji to Papio, proposed phylogenetic divergence of Rungwecebus and Papio followed by mitochondrial introgression, making the kipunji, in a sense, its “own grandpa” (Jaffe and Latham, 1948).

If molecular methods have yet to resolve the kipunji’s ancestry, what can other lines of evidence tell us? McGraw and Fleagle (2006) hypothesized that the moderately deep suborbital fossae and weak maxillary ridges shared by L. aterrimus and Cercocebus agilis represent the primitive African papionin morphology. If so, the kipunji’s resemblance to L. aterrimus in these features is uninformative concerning its ancestry. Additionally, the kipunji cranium offers no evidence of hybrid ancestry. But there is no reason to expect classic hybrid traits (e.g., supernumerary teeth; Ackermann et al., 2006) in a lineage that has had thousands of generations to weed out conflicts between its parental genomes, as witnessed by the fact that the kipunji and other such suspected hybrid taxa have been identified solely on the basis of their DNA (Tosi et al., 2000; Sinha et al., 2005; Chakraborty et al., 2007; Karanth, 2008; Karanth et al., 2008; Osterholz et al., 2008; Burrell et al., 2009; Zinner et al., 2009).

Mangabey biogeography is equally unenlightening as to the kipunji’s potential forbears. Modern L. aterrimus and C. agilis are sympatric throughout much of Central Africa and approximately equidistant from the kipunji’s East African range (Kingdon, 1997). Fossils attributed to Lophocebus from Koobi Fora occur as late as 1.38 Ma and establish this genus’s presence in East Africa well into the Pleistocene (Jablonski and Leakey, 2008). But if the East African relict species C. galeritus and C. sanjei (the latter also endemic to the Udzungwa mountains) are truly descended from a C. agilis–like ancestral population (Grubb, 1978, 1982), Cercocebus ancestry for the kipunji is at least plausible. Whereas DNA, qualitative trait comparisons, and biogeography offer no compelling basis to choose among competing hybridization scenarios, the evidence presented in this study does. The consistent morphometric affinity of the simulated kipunji adults to L. aterrimus strongly favors Lophocebus as the paternal progenitor population of Rungwecebus.

If it falls to geneticists to untangle the evolutionary relationships among African papionins, it is the systematist’s task to wrestle with the taxonomic and biological implications of this increasingly complicated, and perhaps relictive, phylogeny. Several cercopithecoid species are thought to have arisen through intrageneric introgression (Tosi et al., 2000; Chakraborty et al., 2007; Osterholz et al., 2008), and intergeneric hybridization has been reported in both captivity and (less frequently) the wild (Markarian et al., 1972; Chiarelli, 1973; Dunbar and Dunbar, 1974; Jolly et al., 1997). Like the kipunji, the golden and capped leaf monkeys (Trachypithecus geei and T. pileatus) of South Asia are proposed to have arisen through intergeneric hybridization between Semnopithecus and Trachypithecus (Karanth, 2008; Karanth et al., 2008; Osterholz et al., 2008). These cases suggest that natural hybridization has been more important in Old World monkey evolution than previously thought (Arnold and Meyer, 2006) and also raise interesting questions for taxonomists. The taxonomic rank of Rungwecebus rests, in part, on the yardstick by which we measure its morphological divergence, namely morphological distances among other papionin genera. But if members of different cercopithecoid genera are capable of giving rise to viable hybrid populations, we may be forced to reconsider either our taxonomy or our conception of the genus category.

**CONCLUSIONS**

In this study, developmental simulation was used to estimate the adult cranial morphology of **Rungwecebus**
kipunji, “one of the world’s most threatened primates” (Rovero et al., 2009: p 99). The accuracy of our estimate depends ultimately on the degree to which the juvenile kipunji specimen is representative of its species’ morphology. The tests of time and new data will tell how close to the mark our estimate falls, but by the effort, we have gained new insights into kipunji cranial morphology and papionin cranial development.

Cercopithecin developmental vectors were found to be generally similar. Angular differences between papionin species’ vectors are small and even the most different vectors (Mandrillus and Theropithecus) are not statistically distinguishable, although sampling effects cannot be ruled out. The relative uniformity of developmental patterns across the three main papionin clades suggests conservation of an ancestral developmental program from which only the largest species deviate. Simulated kipunji adults based on different developmental vectors were also extremely similar. Deviations of individual simulations from the average estimated shape reflect the influence of different species vectors; however, even the Mandrillus simulation, based on a patently unreasonable model, was phenetically as similar to other estimated adults as to a male mandrill. Simulated kipunji adults are morphometrically closest to Lophocebus aterrimus, but their disjunct distribution and lack of unique shared features are evidence of the kipunji’s distinctiveness. The adult kipunji’s relatively tall, broad neurocranium, broad face, and concave antorbital profile are unique among adult male papionins and identical to traits that distinguish the juvenile kipunji from other M1-stage papionins (Singleton, 2009). This confirms the primacy of early juvenile (pre-M1 stage) morphogenesis in establishing adult cranial shape and suggests that, in most cases, juvenile morphology is reliably predictive of adult form.

The kipunji’s estimated adult cranial morphology is generally similar to that of other mangabeys. Phenetically, it most closely resembles L. aterrimus, but its differences outweigh its similarities. Morphometric distances comparable with those between other papionin genera and a suite of unique cranial features identify the kipunji as a discrete phenon and reaffirm its generic status under current morphological criteria. But if Rungwecebus truly arose through interfgeneric hybridization—just the latest surprise to emerge from the Pando-ra’s box that is the kipunji—our conceptions of papionin genera may be due for amendment.

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LITERATURE CITED


