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Craniomandibular form and body size variation of first generation mouse hybrids: a model for hominin hybridization

Kerryn A. Warren^{a,b}, Terrence B. Ritzman^{a,b,c,d}, Robyn A. Humphreys^{a,b}, Christopher J. Percival^{e,f,g,h}, Benedikt Hallgrímsson^{e,f,g}, Rebecca Rogers Ackermann^{a,b,*}

^aDepartment of Archaeology, University of Cape Town, South Africa

^bHuman Evolution Research Institute, University of Cape Town, South Africa

^cDepartment of Neuroscience, Washington University School of Medicine

^dSchool of Human Evolution and Social Change, Arizona State University, USA

^eDepartment of Cell Biology and Anatomy, McCaig Institute for Bone and Joint Health, Alberta Children's Hospital Research Institute, University of Calgary, Canada

^fThe Alberta Children's Hospital Research Institute, University of Calgary, Canada

^gThe McCaig Institute for Bone and Joint Health, University of Calgary, Canada

^hDepartment of Anthropology, Stony Brook, USA

Abstract

Hybridization occurs in a number of mammalian lineages, including among primate taxa. Analyses of ancient genomes have shown that hybridization between our lineage and other archaic hominins in Eurasia occurred numerous times in the past. However, we still have limited empirical data on what a hybrid skeleton looks like, or how to spot patterns of hybridization among fossils for which there are no genetic data. Here we use experimental mouse models to supplement previous studies of primates. We characterize size and shape variation in the cranium and mandible of three wild-derived inbred mouse strains and their first generation (F₁) hybrids. The three parent taxa in our analysis represent lineages that diverged over approximately the same period as the human/Neanderthal/Denisovan lineages and their hybrids are variably successful in the wild. Comparisons of body size, as quantified by long bone measurements, are also presented to determine whether the identified phenotypic effects of hybridization are localized to the cranium or represent overall body size changes. The results indicate that hybrid cranial and mandibular sizes, as well as limb length, exceed that of the parent taxa in all cases. All three F₁ hybrid crosses display similar patterns of size and form variation. These results are generally consistent with earlier studies on primates and other mammals, suggesting that the effects of hybridization may be similar across very different scenarios of hybridization, including different levels of hybrid fitness. This paper serves to supplement previous studies aimed at identifying F₁ hybrids in the fossil record and to introduce further research that will explore hybrid morphologies using mice as a proxy for better understanding hybridization in the hominin fossil record.

*Corresponding author: becky.ackermann@uct.ac.za (R.R. Ackermann).

Keywords

Gene flow; *Mus musculus*; Introgression; Heterosis; Transgressive traits

Introduction

Hybridization and its phenotypic consequences in primates

Hybridization, the interbreeding between individuals from genetically differentiated lineages, is an important mechanism facilitating evolution (Stebbins, 1959; Lewontin, 1966; Arnold, 1992; Dowling and DeMarais, 1993; Dowling and Secor, 1997; Barton, 2001; Seehausen, 2004; Schwenk et al., 2008; Arnold and Martin, 2009; Feder et al., 2012; Abbott et al., 2013; Dittrich-Reed and Fitzpatrick, 2013; Kronforst et al., 2013). While botanists have embraced hybridization as normal and abundant among diversifying taxa, it is often overlooked in studies involving animals (Mallet, 2005). Despite this, animal hybrids are quite common, with 10% of animal species producing hybrids and with occasional “phylogenetic hotspots” having greater hybridization rates in animals than in plants (Mallet, 2005; Stelkens and Seehausen, 2009). Hybridization occurs across a wide range of mammalian lineages, including (but not limited to) whales (Árnason et al., 1991; Bérubé and Aguilar, 1998); wildebeest (Brink, 2005; Ackermann et al., 2010); bison and domestic cattle (Baranov and Zakharov, 1997); coyotes, wolves, and dogs (Mahan et al., 1978; Vilà et al., 2003; Benson et al., 2012; Khosravi et al., 2013; Monzón et al., 2014); squirrels (Goodwin, 1998; Chavez et al., 2011); and many primate taxa (Jolly, 2001; Detwiler et al., 2005; Arnold and Meyer, 2006; Cortes-Ortiz et al., 2007; Zinner et al., 2011).

In primates, hybridization in the wild occurs within all major lineages. In strepsirrhines, hybridization has been reported among subspecies and species of lemurs, and especially taxa within the genus *Eulemur* (Curtis and Zaramody, 1998; Wyner et al., 2002; Pastorini et al., 2009). In platyrrhines, hybridization has been observed among howler monkeys (genus *Alouatta*; Gregorin, 2006; Aguiar et al., 2007, 2008; Kelaita and Cortes-Ortiz, 2009; Cortés-Ortiz et al., 2015), spider monkeys (genus *Ateles*; Rossan and Baerg, 1977), saddle-back tamarin (*Saguinus fuscicollis*) subspecies (Cheverud et al., 1993; Peres et al., 1996; Kohn et al., 2001), and among different species of marmoset (Coimbra-Filho et al., 1993; Tagliaro et al., 1997; Marroig et al., 2004; Malukiewicz, 2013; Fuzessy et al., 2014; Malukiewicz et al., 2014). Within Old World Monkeys, baboons (genus *Papio*), macaques (genus *Macaca*), and guenon species (genus *Cercopithecus*) exhibit inter- and intra-specific hybridization (Fooden, 1964; Bergman and Beehner, 2004; Wildman et al., 2004; Detwiler et al., 2005; Schillaci et al., 2005; Zinner et al., 2009). Hybridization among ape taxa is less common than in monkeys, no doubt in part because there are simply fewer closely related, sympatric ape taxa. However, hybridization between siamangs and gibbons (genera *Symphalangus* and *Hylobates*, respectively) has occurred in captivity (Myers and Shafer, 1979), and other instances of hybridization have occurred between closely related species both in captivity and in the wild (Montagu, 1950; Brockelman and Srikosamatara, 1984; Marshall and Sugardjito, 1986). Within-genus hybridization among great ape species and subspecies (perhaps even between-genus hybridization) may occur (see discussion in Arnold, 2008), but evidence for it is limited and restricted to the genome (Ackermann, 2010; Prado-Martinez et

al., 2013; but see Ackermann and Bishop, 2010). Furthermore, hybridization between distinct hominin lineages (e.g., Neanderthals, Denisovans, ancient Africa-derived people) has occurred multiple times during the Pleistocene, both outside and within Africa (Patterson et al., 2006; Green et al., 2010; Krause et al., 2010; Reich et al., 2010, 2011; Hammer et al., 2011; Abi-Rached et al., 2011; Meyer et al., 2012; Sankararaman et al., 2012, 2014; Lachance et al., 2012; Wall et al., 2013; Fu et al., 2013, 2014, 2015, 2016; Huerta-Sánchez et al., 2014; Kim and Lohmueller, 2015; Kuhlwilm et al., 2016).

The current literature indicates that there can be considerable variation in the morphological expression of hybridization, with hybrids resembling either parent taxon, being intermediate between the parent taxa (additive outcome), or having morphologies that are extreme or novel (Cheverud et al., 1993; Rieseberg et al., 1999; Stelkens and Seehausen, 2009; Ackermann, 2010). Heterosis or dysgenesis, positive or negative deviations from the intermediate outcome, are terms typically used to describe fitness; for morphology, larger or smaller size is a proxy for increased/decreased fitness. Phenotypic changes in plants, resulting from large scale changes in genomic regulation due to the combining of divergent genomes, is referred to as “genomic shock” (Comai et al., 2003). Similar effects (like the unusual expression of growth-related genes) have also been implicated in the unusually large size of *Peromyscus* (field mouse) hybrids (Duselis and Vrana, 2010).

Although the skeletons of most primate taxa have not been examined for evidence of hybridization per se, wide ranges of morphological variation—especially pelage and body size variation—among primate hybrids have been observed and described (Arnold, 2008; Ackermann, 2010). The work that has been done on the skeleton of primate and other mammalian hybrids shows that hybrids are extreme in size (transgressive) and sometimes express high frequencies of novel traits relative to the parents (Ackermann et al., 2006, 2014; Ackermann, 2010). These include a prevalence of atypical traits associated with a breakdown in the coordination of early development, such as supernumerary teeth and sutural anomalies (Goodwin, 1998; Ackermann et al., 2006, 2014; Ackermann et al., 2010; Ackermann and Bishop, 2010). In terms of craniometric analyses of hybrids, research has focused on understanding heterosis and dysgenesis (i.e., where the hybrids are significantly larger or smaller, respectively, than the additive effect of intermediacy expected based on parental size). Analyses that have examined baboons, gorillas, and tamarins indicate that hybrids (or purported hybrids in the case of the gorillas) are heterotic in the majority of traits tested (Cheverud et al., 1993; Ackermann et al., 2006; Ackermann and Bishop, 2010). The statistical significance of cranial heterosis varies among primate hybrids, with tamarin hybrids exhibiting more significant heterotic cranial traits than baboon hybrids. It is not known how many generations cranial heterosis persists after hybridization has taken place, though there is some suggestion that it might be observable for a considerable amount of time (Ackermann and Bishop, 2010).

The link between unusual hybrid morphology and the phylogenetic distances among parents is difficult to determine. Stelkens and colleagues (2009a) noted a tight correlation between genetic distance and transgressive size in cichlid fish, although this link was most correlated in the F2 generation. Similarly, in the literature of both plants and animal hybrids, transgressive behavioral, morphological, and physiological traits showed a tighter

correlation with genetic distance than phenotypic distance (Stelkens et al., 2009b). This implies that much of the transgression seen in hybrids is the result of complementary gene action or epistasis. However, when studying hybrid skeletal traits, such relationships are less clear. For instance, despite being relatively closely related, the baboon hybrids studied by Ackermann and colleagues (2006, 2014) display high levels of supernumerary dentition and relatively low levels of heterosis.

Primates are excellent models for understanding hominin hybrid morphology, but they have limitations. In particular, slow breeding time, expense, and ethical issues make experimental work with primates unfeasible. Observational data in the wild, or data collected from museum specimens, can be used, but often the degree of introgression is unknown or unknowable. Moreover, collecting skeletal data on wild animals is complicated (and expensive if it involves capture and radiography/scanning). Known genealogies of hybrid primates are rare, with skeletal collections such as the Southwest National Primate Research Center (SNPRC) baboons (Ackermann et al., 2006, 2014) having limited samples beyond the first generation. Additionally, both the SNPRC collection and many museum collections are of crania only; information regarding the postcranial skeleton of hybrids is practically non-existent.

Here we present data obtained from mouse crosses that have been chosen and bred to provide a more comprehensive approach to assessing variation in the hybrid skeletal phenotype. This is the first study from an ongoing project that is examining multi-generation mouse recombinants of several closely related subspecies and two species, designed to generate large samples of mice with various degrees of introgression in the wild. The data generated will ultimately include skeletal cranial and postcranial data, as well as soft-tissue (pelage, muscle) variation, in the context of known genotypes. This paper serves as an introduction to this research, bridging the gap between previous research on the effects of primate hybridization in the skeleton and our larger hybrid mouse research project. Specifically, we are reporting on size, form, and shape variation in the crania of our sub-specific crosses of mice, and comparing these results to previous work on other taxa. Ongoing and future research with these mice will include further in-depth analyses to investigate inter- and intra- specific hybrid morphologies.

Mice as model organisms

Mice are useful model organisms for evolutionary studies (Guénet and Bonhomme, 2003; Berry and Scriven, 2005). They are widely utilized, have available complete genome sequences, can be subject to tightly controlled breeding, and are relatively inexpensive to breed due to short generation times. There is also a diverse and deep literature regarding hybridization and introgression among sub-species of *Mus musculus* (Boursot et al., 1993; Sage et al., 1993; Alibert et al., 1994; Auffray et al., 1996; Renaud et al., 2009, 2012; Teeter et al., 2010) and some research considering hybridization among more divergent species of the genus *Mus* (Forejt, 1996; Montagutelli et al., 1996; Greene-Till et al., 2000; Duvaux et al., 2011). This makes them useful model organisms for addressing many questions regarding the morphology of mammalian hybrids.

This study focuses on the first generation (F₁) hybrids of three different subspecies of *Mus musculus*. Our ultimate goal is to catalogue and interrogate the effects of hybridization in these crosses to more thoroughly interpret the fossil record of human evolution (see Discussion). The divergence of these *Mus musculus* subspecies occurred less than 600 thousand years ago (kya), according to scnDNA (single-copy nuclear DNA) hybridization results (She et al., 1990; Boursot et al., 1993). Wild populations of crosses between these subspecies occur under different scenarios of gene flow. Between *Mus musculus musculus* (which occurs across northern Eurasia, from eastern Europe to Japan) and *Mus musculus domesticus* (in Western Europe, around the Mediterranean), a narrow hybrid zone occurs in Europe (Bonhomme et al., 1987; Teeter et al., 2008; Baird and Macholan, 2012; Bonhomme and Searle, 2012). Male hybrids are typically infertile, following Haldane's Rule, but gene flow still occurs across these groups (Payseur et al., 2004; Teeter et al., 2008, 2010; White et al., 2012). In contrast, the hybrid zone between *M. m. musculus* and *Mus musculus castaneus* (located in southeast Asia) is extensive, with the hybrids being so successful that a hybrid taxon, *Mus musculus molossinus*, has formed (Yonekawa et al., 1988, 2012). As they are not geographically proximate, there is no natural hybrid zone between *M. m. domesticus* and *M. m. castaneus*. Thus, the three different mouse crosses represent potential models for hybridization of recent (mid to late Pleistocene) hominin lineages under three very different scenarios. In these scenarios, the spatial relationships among parent taxa in the wild and outcomes of hybridization differ, possibly as a result of differences in the adaptive success and evolutionary history of the founder strains and associated crosses.

The morphology of *M. m. domesticus* and *M. m. musculus* hybrids has been quantified from inbred laboratory strains and across the hybrid zone in Europe. Much of this research looks at fluctuating asymmetry and developmental stability across this zone and within the hybrids (Leamy, 1984; Alibert et al., 1994; Auffray et al., 1996; Debat et al., 2000). A related study of mandibular size and form in laboratory crosses indicates that hybrids are intermediate in shape, but heterotic, and indeed, transgressive, in size (exceeding that of both parents; Renaud et al., 2009, 2012). Some heterosis has also been shown in other inbred laboratory strains of mice (Chai, 1956a; Carmon, 1963; Leamy, 1982a, b, 1984; Thorpe and Leamy, 1983, 1984; Percival et al., 2016). However, the degree of heterosis in the crossing of inbred laboratory strains appears to vary widely, with the source of this variability sometimes attributed to maternal effects. This pattern is also less clear across the natural hybrid zone (Pallares et al., 2016). This may be because samples collected in natural hybrid zones include multigenerational recombinants that mostly resemble parents, as well as parent strains. However, it is also likely that the natural parental populations are not as inbred as previously studied laboratory mouse populations. In this paper, we explore the skeletal commonalities of body size and cranio-mandibular form among three hybrids from the three parent subspecies described above. We therefore intend to explore potential similarities in heterotic expression among hybrids, and hybrid-parent cranio-mandibular form differences among three crosses from similarly divergent parent groups. This will then be discussed within the context of the previous research that has looked at primate hybrid crania and be used to describe and justify further research.

Materials and methods

Samples and measurements

The sample for the current study comprises mice from three wild-derived inbred *Mus musculus* strains (*M. m. castaneus* [CAST/EiJ, hereafter CAS], *M. m. musculus* [CZECH/EiJ, hereafter CZE], and *M. m. domesticus* [WSB/EiJ, hereafter WSB]), acquired from the Jackson Laboratory (www.jax.org), and their F₁ hybrids (CASxCZE, CASxWSB, and CZE_{Ex}WSB; all crosses were reciprocal). A recent haplotype diversity study of *M. musculus* indicates that wild-derived mouse strains often show a history of hybridization between these three subspecies (Yang et al., 2011) and therefore strains were chosen specifically to minimize any history of mixed ancestry, rather than for other reasons (e.g., locality). Wild-derived strains are comparable with wild-caught populations in being more resistant to infections, in having dominant genetic alleles and phenotypes when crossed with laboratory strains, in behavior (and are therefore used as model organisms for studies in behavioral genetics), and in having a large number of polymorphisms derived from natural populations (Guénet and Bonhomme, 2003). Sample sizes are provided in Table 1. Animals were housed, bred, and sacrificed at the University of Calgary in accordance with relevant federal regulations and approved animal care protocols from the University of Calgary and the University of Cape Town (AC12–0210 and 2012V56RA, respectively).

Following sacrifice, micro-computed tomography (microCT) scans were obtained in the 3D Morphometrics Centre at the University of Calgary using a Scanco vivaCT40 scanner (Scanco Medical, Brüttisellen, Switzerland) at 0.035 mm voxel dimensions at 55kV and 72–145μA. Analyses of body size and cranial form were then conducted. Body size was approximated from lengths of the humerus (most proximal point on the humeral head to distal end of trochlea, following Sargis [2002]), ulna (proximal edge of olecranon process to distal edge of styloid process, following Sargis [2002]), femur (proximal edge of the greater trochanter to the distal end of the lateral condyle), and fibula (distal end of the head to the distal end of the lateral malleolus), all measured from the microCT scans using the Avizo® Fire 8.1.1 software (VSG). Craniofacial form was quantified from 21 three-dimensional landmarks (Table 2, Fig. 1), which were collected using Analyze 3D (version 5.0; www.mayo.edu/bir/) from bone surfaces of micro-CT images of mouse heads. These landmarks were developed in the Hallgrímsson lab and have been used in past publications (see Percival et al., 2016). In addition, 17 mandibular landmarks were collected using Meshlab (v.1.3.2) from mesh surfaces derived from the same CT images and created with Avizo.

Analyses

Pairwise comparisons of limb lengths (*t*-tests: α -level of both $p = 0.05$ and 0.001 were used) were made between CAS, CZE, and WSB (hereafter, referred to as “parent strains” or “parents”) and between CASxCZE, CASxWSB, and CZE_{Ex}WSB (hereafter, referred to as “hybrid strains” or “hybrids”). In addition, limb lengths of each hybrid were compared to those of each of its parents, creating three parent-parent-hybrid sets—i.e., comparisons were made among the following groups: 1) CAS, CZE, and CASxCZE; 2) CAS, WSB, and CASxWSB; and 3) CZE, WSB, and CZE_{Ex}WSB.

To investigate overall cranial and mandibular size, the landmark data were converted to 38 interlandmark distances in the cranium and 21 in the mandible (Fig. 1), which were chosen to provide complete coverage of the cranium and mandible with minimal duplication. Furthermore, these distances, particularly in the cranium, were chosen to allow for greater comparison between that of previous primate hybrid studies. Geometric means for all interlandmark distances were computed separately for the cranium and the mandible, and these geometric means were compared among the combined sample of parents and hybrids using *t*-tests. In addition, the geometric means were compared in each parent-parent-hybrid set.

An analysis of heterosis of overall cranial and mandibular size was also performed using the geometric means of the interlandmark distances. Specifically, the mid-parental value (MPV) was calculated for each pair of parents by computing the average of the bootstrapped means of each of the parents. Mid-parental value 95% confidence intervals (CIs) for each pair of parents were then compared to the means of the respective hybrids. All statistical procedures performed using the interlandmark data were conducted using R, version 3.4.1 (SOM1). Tests of heterosis were also performed for each of the raw interlandmark distances. These tests were conducted in the same way as those for overall cranial size—i.e., MPV 95% CIs for each pair of parents for each interlandmark distance were computed and compared to the means of the respective hybrid.

Significant differences in form were further analyzed by comparing all interlandmark distances among the three parent strains and among the three hybrids. In addition, comparisons were made for each parent-parent-hybrid set. These comparisons were made using pairwise *t*-tests. To compare our data with that of previous hybrid literature, a α -level of 0.05 was used. A more conservative α -level of 0.001 (Bonferroni corrected *p*-value is 0.001 for 38 craniofacial distances, and 0.002 for 21 mandibular distances) is therefore also reported when analyzing form.

To better visualize shape differences between parents and hybrids, principal components analyses (PCAs) for each group-set (three groups each consisting of one hybrid and its two respective parents) were generated in MorphoJ (v1.06a, Klingenberg, 2011) using the Procrustes-aligned cranial and mandibular landmark data. Principal components analyses were used to reduce the Procrustes-aligned coordinates into components representing axes of landmark coordinate covariation, which frequently separated related genotypes in our analyses. It is important to visualize these shape changes across the coordinates that contribute to the greatest amount of shape between individuals, particularly if they are shown to separate groups.

We also explored maternal effect as a potential explanation for heterosis measured in these samples. This was accomplished by analyzing significant differences between the reciprocal hybrids for each cross using *t*-tests, and then comparing the means of these hybrids to a recombinant MPV (female of the maternal strain and male of the paternal strain). Because it is difficult to evaluate ancestry in fossils, this is only briefly addressed in the main text, but the results of these analyses are contained within the Supplementary Online Material (SOM).

Results and preliminary discussion

Comparisons between parent strains

Pairwise comparisons of limb length are shown in the first section of Table 3. Among parents, WSB had the longest forelimb lengths (i.e., humerus and ulna), followed by CAS and CZE, respectively. Similarly, WSB had significantly longer humeri and ulnae than CZE and WSB has the longest hindlimb elements (i.e., femur and fibula), followed by CZE and CAS, respectively. Additionally, CZE and WSB had significantly longer femora than CAS, but no significant difference in femur length existed between WSB and CZE. Fibular length in WSB was significantly greater than in both CZE and CAS, but the differences between CAS and CZE were not statistically significant.

Pairwise comparisons of geometric means of the cranial interlandmark distances (Table 3) indicated significant differences among parents, and between hybrids and their respective parents. Specifically, among parents, WSB had the largest overall cranial size based on geometric mean. Pairwise comparisons of geometric means of the mandibular interlandmark distances are also recorded in Table 3. Among parents, WSB and CZE are not significantly different from each other in mandibular size, yet both differ significantly from CAS.

In analyses of cranial form, the results of the pairwise *t*-tests comparing means among all groups (parent-parent, and parent-hybrid) indicated a similar pattern—i.e., a large majority of the interlandmark distances were significantly different ($p < 0.05$) in each pairwise comparison, and 19/38 were significantly different in all pairwise comparisons (14 with $p < 0.001$). In comparisons among parents, 26 of the 38 interlandmark distances were significantly different in all three comparisons ($p < 0.05$; 22 at $p < 0.001$). In addition to the differences noted above, which vary among all groups, there are specific aspects of skull form that were found to differ among the parent strains specifically (Fig. 2). There are common differences across all groups in both the cranium and mandible and are indicated in the figures ($p < 0.05$; shown in red in Figs. 2–4). In particular, these differences include the anteroposterior length and mediolateral width of the temporal foramen (formed laterally by the zygomatic and squamosal portions of the zygomatic arch and medially by the lateral portion of the squamosal and frontal bones). There are also differences among these strains in the cranial base—i.e., in the anteroposterior length and mediolateral width of the sphenoid wing and the tympanic bulla.

The results of the pairwise *t*-tests comparing means among mandibles of parents indicated a similar pattern—i.e., a large proportion of the interlandmark distances were significantly different ($p < 0.05$) in each pairwise comparison. In comparisons among parents, 11 of the mandibular interlandmark distances were significantly different in all three comparisons ($p < 0.05$; 7 at $p < 0.001$). Although these measurements are found throughout the mandible, they are predominantly located in the anterior portion (Fig. 2). In the mandible, parent strains differ in the superoinferior height of the coronoid and condylar process, as well as in the anteroposterior length and superoinferior height of the entire mandibular corpus and incisor alveolus. Furthermore, there is no difference among parent groups in the distance between the molar tooth row and the base of the incisor crown on the superior aspect of the mandible.

Comparisons between the CASxCZE hybrid and their parent strains

Table 4 summarizes the percentage of measurements whereby the hybrids fall intermediate, larger, or smaller than the two parents, and the proportion of measures which were transgressive, heterotic, or dysgenetic in the hybrids compared with the parents. Both postcranial and cranial comparisons between hybrids and parents showed heterosis in hybrids. Postcranially, the hybrids had significantly longer long bones than both parents in all measures. The geometric means for both the cranium and mandible were also significantly larger in hybrids compared with the parents. In comparisons of cranial form of CASxCZE to its parents, 30/38 interlandmark distances were larger than that of both parents and 22 measures were significantly different and larger than in both parents (12 at $p < 0.001$). Two distances were significantly smaller in CASxCZE compared to the parents ($p < 0.05$; one at $p < 0.001$: NAS-IOS, which describes facial width). In mandibular comparisons of CASxCZE to its parents, 11/21 interlandmark distances were significantly different and larger than in both parents at $p < 0.05$ (eight at $p < 0.001$). Thirty-one of the cranial MPV CIs (82%) were below the CASxCZE mean, and four of the intervals were above the mean (11%). Seventeen (81%) of the mandibular MPV CIs were outside the CASxCZE mean (16 of these were below). (See SOM2 for the calculated 95% CI of the combined parent strains, and SOM3 for this interval when the parental sexes are recombined separately).

Comparisons between the CASxWSB hybrid and their parent strains

Similar to that seen in the CASxCZE hybrids, both postcranial and cranial differences were observed in the CASxWSB hybrids and their parents. Postcranially, the hybrids again had significantly longer long bones than both parents. Among all the hybrids, CASxWSB had the largest geometric means for cranial size and was significantly larger than its parent strains. The geometric mean for the mandible was significantly different between the hybrid and CAS parent (larger in the hybrid), but not between the hybrid and WSB. The similar mandibular size of WSB and CASxWSB may be because WSB had the largest overall mandibular size based on the geometric mean of the parents (followed by CZE and CAS, respectively), while CASxWSB had the smallest mandibular size of the hybrids (CZExWSB had the largest).

In the comparison of cranial form of CASxWSB to its parents, 21/38 distances were significantly larger than in both parents (12 at $p < 0.001$). In this comparison, two distances were significantly smaller in the CASxWSB compared to both parents (at $p < 0.05$; one at $p < 0.001$: PSA-MPP, which describes palatal width). In the mandibular comparison of CASxWSB to its parents, only three distances were significantly larger than in both parents at $p < 0.05$ (none at $p < 0.001$), although 17 were significantly larger than in CAS (the smaller parent). Thirty-four of the cranial MPV CIs (89%) were outside and below the mean of CASxWSB, and three were outside and above. Nineteen (90%) of the mandibular MPVs were outside and below the mean of CASxWSB. (See SOM2 and 3 for the calculated 95% CI of the combined parent strains).

Comparisons between the CZExWSB hybrid and their parent strains

Per the other crosses, both postcranial and cranial differences were calculated. Postcranially, the hybrids again had significantly longer long bones than both parents. Furthermore, the

hybrids displayed significantly larger cranial and mandibular size compared with both parents. In the comparison of cranial form of CZExWSB to its parents, 22/38 of the interlandmark distances were significantly longer than in both parents at $p < 0.05$ (12 at $p < 0.001$). However, two of the interlandmark distances were significantly smaller in CZExWSB compared to both parents (at $p < 0.05$, none at $p < 0.001$; OAS-OA and NAS-IO). In the mandibular comparison of CZExWSB to its parents, 9/21 of the interlandmark distances were significantly larger than in both parents at $p < 0.05$ (six at 0.001). Thirty-four of the 38 cranial MPVs (89%) were outside and below the mean of CZExWSB. Seventeen (81%) of the 21 mandibular MPVs were below of the CZExWSB hybrid mean. (See SOM2 and 3 for the calculated 95% CI of the combined parent strains).

Comparing the three hybrid-parent datasets to identify common trends

In all parent-parent-hybrid sets (i.e., comparisons of hybrids to their respective parents), the hybrids had significantly longer long bones than parents for all the bones measured.

In the analyses of size heterosis for the geometric means of all cranial interlandmark distances, the parental MPV CIs fell below the hybrid means in all cases. This is expected as hybrids possessed significantly larger cranial geometric means than their respective parents in all comparisons.

For the pairwise t -tests (Table 3), across all comparisons of hybrids to their respective parents, the results indicated that a majority of interlandmark distances in the hybrids are significantly different ($p < 0.05$) from their respective parents. Figure 3 shows that in two additional measurements, the three hybrids are significantly larger (at $p < 0.05$) than their respective parents (in measurements associated with the basicranium). At $p < 0.001$, all three hybrids are transgressive (greater than both parents) in the length of the palate. As shown in Figure 3, there are fewer differences between hybrids and parents than there are among the parents. It should be noted, however, that this result may be due to the fact that the patterns of differences between hybrids and parents are not identical across the three parent-parent-hybrid sets. In other words, the similarities/differences between any one hybrid and its respective parent strains may not be the same as those between another hybrid and its respective parents, resulting in a relative dearth of measurements that are different in all parent-hybrid comparisons.

In 13 of the total 38 measurements, all the hybrids were significantly larger than either parent (at $p < 0.05$, nine at $p < 0.001$). Figure 4, which illustrates the consistent differences between each hybrid and its respective parents, demonstrates that measurements are generally either heterotic and/or significantly larger in hybrids relative to parents generally. This corroborates the results of comparisons of overall cranial size by demonstrating the overall larger size of each hybrid relative to its parents. The only cranial regions in which hybrids are not larger than parents are the anteroposterior length and mediolateral width of the frontal bone (especially in the more posterior portion), the superoinferior height of the posterior part of the neurocranium, and the mediolateral width of the palatine bone.

For the mandible, the results are similar to that seen in the cranium, with pairwise t -tests indicating that a majority of interlandmark distances in the hybrids were significantly

different from their respective parents. Figure 3 shows that in five measurements, the three hybrids are significantly different from their parents ($p < 0.05$, none at $p < 0.001$) in measures of the tooth row and anterior mandible especially. If we include measurements in which the hybrids were significantly different from the parents in four or five of the six potential comparisons, the measurements are seen throughout the mandible, possibly reflecting the overall mandibular heterosis. In the majority of these measurements, the hybrids are greater in size than one or both parents. None of the interlandmark distances were significantly smaller in any of the hybrids compared to both of their parents ($p < 0.05$).

In analyses of cranial heterosis, MPVs for the majority of the interlandmark distances were lower than the means of the respective hybrids. Notably, MPVs for 28 of the interlandmark distances were outside and below the respective hybrid means in all parent-parent-hybrid sets. This is a strong indication that hybrids are larger than parental midpoints in the majority of the cranial measurements (Fig. 4).

Similarly, in analyses of mandibular heterosis, MPV CIs for 15 (71%) of the interlandmark distances were outside and below the confidence intervals of the respective hybrids in all parent-parent-hybrid sets (Fig. 4). There were also fewer differences between hybrids and parents in the mandible, and the specific differences that are unique to the hybrid-parent comparisons resided in the mandibular corpus near the molar tooth row and in the anteroposterior length and superoinferior height of incisor alveolus.

Figure 5 shows the PCAs of the Procrustes-aligned cranial landmark data. In all three analyses, principal component (PC) 1 (between 36.8% and 43.9% of the total variance of each PCA) indicates that the largest proportion of variance in cranial shape separates the parental groups. On plots of specimens along PC1 and PC2, the hybrids were largely intermediate, although overlapping more with one of the parents along PC1. Principal component 1 largely reflects shape differences between parent strains, which appear to differ in relative cranial length and neurocranial height in all three PCAs (the grey wireframe corresponds to the more negative side of the axis and the black to the more positive side). This agrees somewhat with the pattern seen in the analyses of linear distances, although the features noted in our PCA analysis are obscured by the large differences in absolute skull size noted between hybrids and parents. In each PCA, the hybrid group is more extreme along PC2 (between 11.9 and 16.4% of the total variance), with the parents overlapping greatly at the other extreme. Shape variation along PC2 is associated with differences in relative snout/palatal length, facial/anterior-temporal width, neurocranial height, and the width of cranial base in all three PCAs, where hybrids appear to have relatively longer snouts, thinner faces, and shorter crania. This differs from the comparisons of linear distances, possibly because distances related to snout length differed in all pairwise comparisons.

Figure 6 shows the PCAs for the Procrustes-aligned mandibular landmarks. Similar to the pattern seen in the crania, plots of PC1 and PC2, which represent the majority of the variance (PC1: between 42.8% and 47.2%), show the parental strains separated along PC1, with hybrids found intermediately. In all three crosses, the shape differences associated with PC1 and PC2 are found across many of the mandibular landmarks, but there are stronger

shape differences in coronoid height and position (the grey corresponding to the more negative part of the axis). These are features that differed between all parents in comparisons of interlandmark distances, which are measurements of size, while these PCAs are derived from landmark coordinates after removing size. However, there are other differences unique to each parent cross, such as along the molar row between CAS and WSB and CAS and CZE and along the inferior border between CAS and WSB and CZE and WSB. Along PC2 (between 12.6% and 19% of variance), the hybrid is a clear extreme in the CASxCZE cross and the CZE $\times$ WSB cross, but in the CAS $\times$ WSB cross, there is a lot more overlap between the hybrids and parents. Variation in posterior and inferior profiles of the mandibles seem to be the shape change most prevalent in all three crosses along PC2, with differences along the molar alveolar region also prevalent. Relative molar length was a feature observed to differ between hybrids and parents in mandibular form.

Exploring maternal effect as an explanation for hybrid heterosis

Heterosis effect in these analyses may be due, in part, to a maternal effect. In particular, in the case where the larger strain is the dam, the hybrid may be larger than the average for the combined hybrids. In comparing the hybrids with the reciprocal cross, there are significant differences between a number of the measures (see SOM4 for a summary of these findings).

Comparing CASxCZE and CZE $\times$ CAS (the dam is of the first strain named), 48% of the mandibular measures and 26% of the cranial measures were significantly different (SOM4). Furthermore, the mandibular geometric mean was significantly different between the crosses, with CZE $\times$ CAS being larger (CZECHI, the dam, is the larger of the parents). CZE $\times$ CAS was larger on average in the cranial geometric mean as well, but this was not significant. Similarly, 38% of mandibular measures and 26% of cranial measures were significantly different between CAS $\times$ WSB and WSB $\times$ CAS, with the latter significantly larger in mandibular geometric mean (WSB is the larger of the parents). However, in the comparison of CZE $\times$ WSB and WSB $\times$ CZE, 19% of the mandibular measures and 47% of the cranial measures were significantly different. WSB $\times$ CZE is significantly larger in cranial, and larger but not significantly so in mandibular, geometric mean. These results indicate a maternal effect, but one that is not consistent in affected regions or measures. This likely reflects variation, and especially size differences, between the parents. In analyzing heterosis in these separate groups, it is worth noting that hybrids were heterotic in 67%—86% of cranial measures (dysgenesis in 5–10%) and 79%—89% of mandibular measures (dysgenesis in 0–5%). All hybrids were heterotic in both cranial and mandibular geometric means.

Discussion

Body size and limb length

The results presented here show that the F₁ hybrid mice have longer limbs than their respective parents. This result was consistent across all parent-parent-hybrid sets. Taken together, and based on previous definitions (Chai, 1956b), these results suggest that the F₁ mouse hybrids examined here can be considered transgressive (more extreme; in this case, larger than parents) in terms of the postcranial skeletal elements measured here. The results

of comparisons of body size (limb length) and overall cranial size—i.e., that hybrids, by and large, have larger crania and longer limbs—are consistent with previous studies of mouse hybrids (Eaton, 1953; Chai, 1956b; Carmon, 1963; Leamy, 1982a; Thorpe and Leamy, 1983; Kurnianto et al., 1999; Pallares Amaya, 2015; Pallares et al., 2016; Percival et al., 2016). It is important to note that although these previous studies, like the present study, used strains representing subspecies of *Mus musculus*, not all strains (or even subspecies) included in the previous work are identical to those used in this study.

It is possible that this effect may be partially explained by restored heterozygosity associated with hybridization between inbred parent strains; inbreeding in the parent strains can make strains smaller on average (Bruell, 1964; but see Lynch, 1977) with hybridization returning the morphology to a natural size (Lerner, 1954; Barnett and Scott, 1964; White, 1972). This may explain why the mice used in this study are more extreme in morphology compared with the natural population hybrids (Pallares et al., 2016). However, studies with both inbred and non-inbred mice (derived from natural populations) show that hybridized (outbred) mice derived from *M. m. musculus* and *M. m. domesticus* are more extreme in both cases (Lynch, 1977; Renaud et al., 2009). The less extreme morphology seen in natural populations may be masked by sampling individuals that have extensively recombined with parents.

These results (of hybrid heterosis) are also generally consistent with previous studies of primates, which have noted transgression in both size and other phenotypic traits for a number of primate hybrids (Kohn et al., 2001; Ackermann et al., 2006, 2014; Kelaita and Cortes-Ortiz, 2009; Ackermann, 2010). In the most directly comparable study, when considering postcranial morphologies of the hybrids of two tamarin crosses (*Saguinus fuscicollis illigeri* x *Saguinus fuscicollis lagonotus* and *S. f. illigeri* and *Saguinus fuscicollis leucogenys*), significant heterosis was found in six and 15 out of 50 postcranial measurements, respectively (although the hybrids were heterotic, albeit not significantly so, in 45 and 44 traits, respectively).

Craniomandibular form

Similarly, based on the results of the analyses of cranial size, F₁ mouse hybrids have significantly larger crania than their respective parents; this finding is shared across all parent-parent-hybrid sets. Similar results are seen in the mandible, although in the case of comparisons between the smaller of the three hybrids (CASTxWSB) and its larger parent (WSB), mandibular size was not transgressive. It is worth noting that in this instance, the mandibular geometric mean of CASTxWSB was similar to the larger parent but larger than the midparental mean, which still suggests heterosis. Hybrids between wild-derived strains observed in other studies show similar patterns (Leamy, 1982a; Thorpe and Leamy, 1983; Leamy and Thorpe, 1984; Leamy, 1992; Percival et al., 2016), with F₁ hybrids having larger overall cranial size than parent (i.e., founder) strains. Other studies that have looked at wild-derived inbred strains representing the above subspecies and their hybrids in Europe have similarly shown marked heterosis in cranial, mandibular, and dental metrics (Alibert et al., 1997; Debat et al., 2000; Renaud et al., 2009, 2012). Studies of natural hybrid zones, however, are more ambiguous. Pallares and colleagues (2016) found that measures for craniofacial and mandibular size show a weak trend, with samples increasing in size towards

the center of the hybrid zone, but that this pattern is non-significant. This contrast may be due to samples comprising a mixture of parents, F₁ hybrids, and multigenerational recombinants.

Increased cranial size in hybrids is also consistent with previous studies of primate crania (Cheverud et al., 1993; Ackermann et al., 2006; Willmore et al., 2009), although levels of cranial heterosis/transgression in primate hybrids appear variable among different species. In saddleback tamarins, two groups of hybrids from different subspecies of saddleback tamarins show a similar pattern of heterosis, although they differ in extent (Cheverud et al., 1993). *Saguinus fuscicollis illigeri* x *S. f. lagonotus* hybrids displayed cranial measures that were significantly larger than both parents for two traits (in the basicranium and palate), with 56% of measures heterotic relative to a mid-parental value and hybrids generally displaying extreme size and size-related shape relative to parents. While these hybrids closely resembled their larger parent, lack of size additivity in this hybrid was noted. Hybrids between *S. f. illigeri* and *S. f. leucogenys* had cranial measurements that were larger than either parent for the majority of traits, hinting at heterosis as well. However, few of these differences were significant, possibly because of small sample size.

For baboons, hybrids between olive and yellow baboons appeared transgressive, or significantly larger than both parents, in one metric signifying basicranial length (Ackermann et al., 2006). The majority of measurements (78%) were larger than the mid-parental value (heterotic), although only 8% were significantly so, with no significant dysgenesis (smaller than the MPV). Thus, both the excess size in hybrid primates for many measurements, as well as transgression in the basicranium, is consistent with our results from comparisons of cranial form in hybrid and parent mice. For mice, only one measure, describing the width of the nasal bones, displayed significant dysgenesis in all three mouse crosses.

Consistency of heterosis and transgression in hybrids

Probably the most significant result of this study is that the morphological trends of large size and extreme shape, and the pattern of size/shape variation, are consistent across all three mouse hybrid groups, despite the very noticeable differences in natural hybrid zones and hybrid success among these three subspecies (Boursot et al., 1993; Bonhomme and Searle, 2012). This adds to previous work on mice and primates (where crosses were between only two taxa and not three) by showing that crosses between similarly divergent organisms, that nonetheless have very different contact scenarios, result in comparable patterns of transgression. These consistencies of form include more extreme size in hybrids, as well as relatively longer snouts in the hybrids, as reflected in significant differences between them and their parents in facial and palatal measurements. Hybrids also display larger cranial base and cranial vault measures, as reflected in significant differences in measures of the petrous temporal bone and parietal bone, among other measurements.

In the PCA of Procrustes coordinates (which removes overall scale), the axis of greatest variance (PC1) separates parents, with hybrids intermediate. Hybrids are distinguished from parents along PC2 (with the exception of the CASTxWSB mandibular analysis). The shape changes associated with hybrids along PC2 include shorter relative neurocranial height,

longer relative snout length, and molar rows compared with parents. These relationships are consistent with what is observed in the interlandmark distance hybrid-parent comparisons, though the latter are somewhat obscured by the inclusion of size in those comparisons.

Although other studies of the crania of mice and primates have produced similar results, there are some differences in the degree and pattern of expression of heterosis/transgression. Such variability in the extent to which hybridization produces transgressive or heterotic traits may be explained by different mechanisms. First, there may be inbreeding depression of the inbred parental genotypes of laboratory studies of mice, combined with restored heterozygosity in the hybrids. While mouse hybrids in the wild do not show the same level of size transgression in the mandible and cranium, they do exhibit extreme size relative to the parents (Alibert et al., 1994, 1997; Macholan, 1996; Renaud et al., 2009; Pallares et al., 2016). However, this has only been demonstrated in populations representing one of the crosses explored here (CZECHI-*M. m. musculus* and WSB- *M. m. domesticus*), which form a hybrid zone in Europe. Furthermore, observations on unknown-cross wild hybrid populations are complicated by the mixed nature of hybrid zones (e.g., parents, F1s, F2s, backcrosses) and are therefore not directly comparable to the results demonstrated here. Second, differences in phylogenetic distance/divergence in the parents may allow for greater accumulation of divergent underlying mechanisms determining size in the more divergent crosses, which may impede additivity expected in the offspring. This suggestion will be explored in future studies, using F₁ crosses between *Mus spretus* and *M. musculus* species combined with the subspecific crosses produced for this study.

There are myriad possible explanations for increased body and cranial size in hybrids vis-à-vis parents. At a very basic level, variation in the size of skeletal elements is due to differences in the rate, timing, and pattern of growth (Lieberman, 2011). Early in development, size differences can be established by the size of the initial cell condensations (which can differ based on the initial number of cells, the timing of initiation and termination of cell condensation, and the rate of cell division; Atchley and Hall, 1991) or by differences in regulatory genes that affect developmental patterns and/or the differentiation and regulation of cells that form the skeletal element in question (e.g., osteoblasts, osteoclasts, and chondrocytes). Later in development, size differences may arise via differences in the rate or duration of growth—i.e., larger size may be due to a longer growth period and/or a faster rate of growth. Although they were performed on different strains than those investigated here, previous studies of mice have shown that hybrids exhibit increased growth rate (Eaton, 1953; Chai, 1956a; Carmon, 1963), and numerous studies of laboratory rodents have established that hybrids have longer lifespans than parents (see Myers [1978] for a review). A systematic increase in growth hormone may underlie this increased growth rate. Although these issues are largely outside the scope of the current study, ongoing work by this group will address the genetic and hormonal bases of size differences between hybrids and parents.

Maternal effect is often seen as another factor in heterosis: where the maternal lineage contributes more greatly to the morphology due to exposure to the dam's hormones in utero and during lactation. While maternal effect appears to affect the morphology of the mice

studied here, transgression and heterosis beyond this effect are still persistent, with hybrids, on average, being significantly larger than both parents in multiple measures.

We propose three non-mutually exclusive explanations for the general consistency of the results presented here. First, as a result of heterosis, all hybrids may possess relatively large masticatory muscles, which in turn cause enlargement of the aspects of the cranium to which this musculature attaches, including the ventral and dorsal aspects of the facial skeleton, temporals, and parietals. Second, the consistency among these patterns may also result from constraint on certain aspects of cranial anatomy, even under the pressures of restored heterozygosity and resultant heterosis. This could be related to the need to maintain functionality in the important sensory and mechanical systems housed in the cranium (e.g., vision, olfaction, mastication). Third, the common effects of hybridization on the cranium may be due to the fact that hybridization may perturb the normal process of cranial ontogeny and, in particular, may modify the duration of specific post-natal ontogenetic phenomena. For example, the increased length of the facial skeleton may be explained by an increase in the duration of facial growth, which occurs later in post-natal ontogeny (e.g., compared to brain growth) and results in longer, more anteriorly projected faces. Increases in the duration of facial growth may also help explain the presence, in many primate hybrids, of supernumerary teeth—i.e., extended tooth morphogenesis could result from (or be correlated with) abnormally long periods of facial growth (Ackermann, 2007; Ackermann et al., 2014).

Large size and unusual morphologies are often explained by epigenetic effects early in development: perturbed growth hormone expression or a breakdown in chromatin integrity (Michalak, 2009; Duselis and Vrana, 2010; Wolf et al., 2014). Presently, we do not have the data necessary to test the relative veracity of each of these explanations.

Implications for hybridization in hominins

Despite the prevalence of gene flow among primates, the existence of hybridization among fossil hominin taxa has, until recently, been hotly debated (Jolly, 2001; Harvati, 2003; Currat and Excoffier, 2004; Reed et al., 2004; Ackermann, 2005; Harvati et al., 2005; Smith et al., 2005; Zilhão, 2006; Klein, 2008). It is only with the partial genome sequencing of Neanderthal and Denisovan autosomal DNA (Green et al., 2010; Reich et al., 2010, 2011) that researchers have definitively demonstrated that human ancestors leaving Africa in the late Pleistocene interbred with other archaic species. Gene flow also occurred between anatomically modern humans and other (yet unknown or not yet sequenced) hominins (Wall et al., 2009; Hammer et al., 2011; Schlebusch et al., 2012). Clearly these ancient DNA (aDNA) analyses have improved our ability to identify past hybridization events, thereby greatly improving our understanding of the evolutionary history of our species. However, aDNA is currently limited to relatively recent lineages and is subject to the idiosyncrasies of preservation. Considering the prevalence of hybridization among contemporary primate taxa, and the genetic observations that gene flow occurred in the past for many other primate taxa, it is likely that hybridization events occurred multiple times during the evolution of our species (Jolly, 2001; Arnold, 2008; Ackermann, 2010), but remain undetectable via aDNA routes.

Empirical observations of a number of primate and other mammalian taxa have shown that there are unifying phenotypic characteristics that identify hybrids, including increased variation and non-metric traits and unusual morphologies (Ackermann et al., 2006, 2010, 2014; Ackermann, 2009, 2010; Ackermann and Bishop, 2010). Based on these studies, Ackermann and colleagues (Ackermann, 2010; Eichel, 2014; Eichel and Ackermann, 2016) suggest a number of hominin specimens that may be hybrids or recombinants. Specimens such as Krapina 58, Skhul IV mandible and maxilla, Amud I, and Qafzeh 9 and 11 show dental anomalies that range from rotated premolars to dental crowding and reduced or enlarged dentition (Ackermann, 2010). Furthermore, these early *Homo sapiens* and Neanderthals have large nasal cavities, which may further hint at hybridization (Eichel and Ackermann, 2016). Similarly, Pestera cu Oase 2, from Romania, has large third molars outside the range of variation in contemporary samples (Tattersall and Schwartz, 1999; Soficaru et al., 2006; Rougier et al., 2007; Ackermann, 2010). One of the specimens associated with Oase 2, Oase 1, has recently been shown to have a Neanderthal ancestor between four and six generations previously, retaining around 6–9% Neanderthal DNA, and autosomal segments that are largely intact and therefore show little recombination (Fu et al., 2015).

Autosomal segments in non-Africans today suggest that interbreeding occurred between 37 and 86 ka and likely 47–65 ka (Sankararaman et al., 2012). Furthermore, new estimates of divergence indicate that Neanderthals and humans interbred as early as 270 ka (Prosth et al., 2017). The date of the Pestera cu Oase mandible (~39–41 ka) is at the recent end of these estimates (and outside of the more restricted range). Moreover, Oase 1 is later than previous estimates of hybridization based on the genome from the Ust’Ishim femur, where autosomal mutation rates estimated Neanderthal gene flow occurred 7,000–13,000 years before the individual lived (i.e., between 50 and 60 ka; Fu et al., 2014). Oase 1 may be from a population that did not contribute DNA to future Eurasian populations; nonetheless, the picture emerging from these studies and others is consistent with a scenario of repeated hybridization events, some of which were fairly recent (Ackermann et al., 2016).

Moreover, despite the proposed prevalence of evidence for cultural interactions between Neanderthals and modern humans in Europe, East Asians share more Neanderthal genetic ancestry than Europeans (Meyer et al., 2012; Wall et al., 2013; Vernot and Akey, 2015). While there is evidence that selection has played a crucial role in the survival and removal of Neanderthal alleles in present-day populations (Sankararaman et al., 2014; Vernot and Akey, 2014), neither selection nor population size can adequately explain the higher Neanderthal ancestry in East Asians (Kim and Lohmueller, 2015; Vernot and Akey, 2015). Again, this is consistent with a complex history of admixture between modern humans and Neanderthals, with multiple pulses of gene flow, though it is also possible that European admixture has been masked by interbreeding with another ancestral population that is yet unknown (Kim and Lohmueller, 2015; Vernot and Akey, 2015).

Given that hybridization in the human fossil record is likely to be much more prevalent than previously realized, with its traces inaccessible through aDNA for most of our evolution, it is imperative that we continue to examine and quantify its phenotypic consequences. The hybrid mice studied here represent crosses between closely related populations, similar to

what we see for these hybridizing hominins. This is important because the morphological expression of hybridization differs depending on the phylogenetic distance between the parental groups (i.e., hybrids between closely related populations exhibit additivity and heterosis, while those between more distantly related populations can display dysgenesis or abnormalities; Ackermann, 2010).

In this context, the results presented here suggest general trends that might be useful for identifying hybrids in the human fossil record. The most obvious and consistent hybrid F_1 phenotype is for cranial size and limb length that is greater than the larger parent, but with unusually high levels of heterosis in certain features such as molar length. If we extrapolate this pattern onto modern humans and Neanderthals, an F_1 hybrid cranium could potentially be as large as, or larger than, a Neanderthal cranium, with associated size-related shape differences, but the overall cranial morphology could still more closely resemble the smaller parent, the human, or with isolated heterotic features. This description can fit a number of fossil hominins in Europe that have previously been classified as modern humans, including Pestera cu Oase 2, which is described as having traits more associated with modern humans but with a large facial skeleton (Rougier et al., 2007). Since the cranium is associated with the known-hybrid Oase 1 mandible (Fu et al., 2015), this re-classification seems quite reasonable.

Although non-metric trait variation was not examined here (and indeed it is unlikely that dental trait variation in mice will provide a good analog for hominins), other studies have suggested that atypical non-metric dental variation, especially if bilateral, is also indicative of F_1 and other hybrids (Ackermann et al., 2006, 2014; Ackermann, 2010). Both Oase 1 and 2 are also described as having unusual (and bilateral) non-metric traits, such as extremely large molars that become larger and display greater occlusal surface size, distally along the tooth row (Trinkaus et al., 2003; Rougier et al., 2007; Ackermann, 2010). Rougier and colleagues (2007: 1169) noted the shared unusual traits between Oase 1 and 2, going as far as to say there is a “close affinity between them” and that the unusual mosaic of features could represent admixture between modern humans and Neanderthals (see also Ackermann, 2010).

Both Oase 1 and 2 have molars that exceed the range of variation for both Neanderthals and Upper Paleolithic modern humans. While transgression in the F_1 mice appears to be more widespread (although it is also recorded in the mandibular tooth row), it is likely that in subsequent generations of hybrids (Backcrosses and F_2 s), a breakdown in the covariation of these traits could occur. This may result in transgressive traits that occur within an otherwise parental form. This breakdown in integration and modularity has been demonstrated in the mandible of mice hybrids and several other species (Renaud et al., 2012) and will be further explored within the context of this research project in subsequent papers. Indeed, the metric evidence presented here, in combination with previous non-metric evidence, is pointing to a clearer picture of what we should expect a hybrid to look like in the human fossil record.

Finally, the use of inbred versus wild-caught mice as a model requires some consideration when applied to the fossil record. It is possible that the pattern seen in the mice is exaggerated owing to inbreeding depression (which is common in inbred strains), with

outbreeding producing an exaggerated heterotic expression due to greater heterozygosity (and therefore fitness). Because of this, we could argue that any hominin hybrids should be expected to have less pronounced hybrid morphologies. However, given the evidence for inbreeding in both early modern human populations (Henn et al., 2015) and within Neanderthal populations (Castellano et al., 2014; Prüfer et al., 2014), it could be argued that restored heterozygosity in their hybrids could play a comparable role in generating skeletal variation. However, it is very likely that the heterotic effects will not be as exaggerated in hybrids of these hominins as seen in these laboratory mice.

Further research

The morphological trends seen across the three mouse hybrids are shared, despite the very noticeable differences in natural hybrid zones and hybrid success (Boursot et al., 1993; Bonhomme and Searle, 2012). This result, along with observational studies on other mammalian hybrids (Cheverud et al., 1993; Kohn et al., 2001; Ackermann et al., 2006; Kelaita and Cortes-Ortiz, 2009), provides compelling evidence that these trends can be extrapolated onto other mammalian species. However, certain questions remain that were not addressed in this analysis.

First, this analysis only applies to F₁ hybrids; it is not known whether a comparable morphological pattern will exist in multi-generational recombinants. To examine this, larger studies and systematic examinations of F₂ and backcrossed mouse samples are currently underway. Second, limited information is available about how and whether genetic and temporal divergence affects the morphology of hybrids. Researchers have suggested that larger divergence time may actually “lessen” the predominance of transgressive morphologies (Stelkens and Seehausen, 2009). Efforts are also currently underway to hybridize a more divergent mouse species of the same genus (Boursot et al., 1993; Auffray and Britton-Davidian, 2012; Bonhomme and Searle, 2012)—*Mus spretus*—with the *Mus musculus* taxa to test whether phenotypic effects are comparable in crosses between more divergent taxa.

Finally, as discussed above, it is unclear how the homozygous (inbred) nature of the parent samples used here might have affected our results. Inbreeding depression, which has been shown to reduce fitness (litter size, survival and overall body weight), will ultimately affect the comparisons between the inbred parents and the F₁ hybrids, making it difficult to deduce the extent to which the transgressive heterotic size of observed hybrids is due to the hybridization between the disparate groups, or due to the inbreeding depression within the parents (and restored heterozygosity in the hybrid offspring). Research comparing levels of heterosis/transgression between our F₁ mice and F₁ hybrids of wild-caught mouse populations is currently underway. These future analyses will undoubtedly provide a greater understanding of the morphological variation expressed in hybrids and hybrid zones.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Diagrams illustrating landmarks captured and measurements calculated in the cranium and mandible. Landmark abbreviations can be found in Table 2.

Figure 2.

Diagrams illustrating the results of pairwise significance testing for each measurement on the cranium and mandible between parental strains. In Figures 2–4, red interlandmark distances are significantly different in all 12 parental and hybrid comparisons at $p < 0.05$ (therefore all groups demonstrate significant differences from one another in those measurements). Measurements in blue in this figure are significantly different in all three parent-parent comparisons at $p < 0.05$. Black lines refer to interlandmark distances which are not significantly different among strains in the above comparisons.

Figure 3.

Diagrams illustrating the results of pairwise significance testing for each measurement on the cranium and mandible for hybrid-parent comparisons. In Figures 2–4, red interlandmark distances are significantly different in all 12 parental and hybrid comparisons at $p < 0.05$ (therefore all groups demonstrate significant differences from one another in those measurements). Measurements in blue are significantly different in all six hybrid-parent comparisons at $p < 0.05$. Black lines refer to interlandmark distances which are not significantly different among strains in the above comparisons.

Figure 4.

Figure illustrating significant heterosis and transgressive measurements in the cranium and mandible. Measurements where Mean Parental Value (MPV) is below the hybrid means are in red (i.e., heterotic). Measurements that are significantly larger ($p < 0.05$) in all three hybrids relative to their parents in blue (i.e., transgressive). Measurements that are significantly smaller ($p < 0.05$) in all three hybrids relative to their parents are in purple (i.e., dysgenic). Black lines refer to interlandmark distances which are not significantly heterotic, dysgenic or transgressive in all three hybrids relative to parents.

Figure 5.

Principal components analyses on the Procrustes-aligned cranial landmarks for the a) CASxCZE cross, b) CASxWSB cross, and c) CZExWSB cross. Specimens are plotted along PC1 and PC2, where CAST specimens are light blue, CZE are green, WSB are dark blue, and F1 hybrids are in red. Wireframe diagrams showing shape change along PC1 and PC2 are on the right of each PC plot.

Figure 6.

Principal components analyses on the Procrustes-aligned mandibular landmarks for the a) CASxCZE cross, b) CASxWSB cross, and c) CZExWSB cross. Specimens are plotted along PC1 and PC2, where CAST specimens are light blue, CZE are green, WSB are dark blue, and F1 hybrids are in red. Wireframe diagrams showing shape change along PC1 and PC2 are on the right of each PC plot.

Table 1.

Sizes of the parent and F₁ hybrid samples used in this study.

Sample	Cranial	Mandibular	Hindlimb	Forelimb
CAS	49	30	30	30
CZECHI	28	30	29	26
WSB	49	29	30	30
CASxCZE	50	30	31	30
CASxWSB	50	30	36	30
CZEExWSB	37	29	30	30

Table 2.

Descriptions and abbreviations for the landmarks used in this study.

Abbreviation	Description
Cranial landmarks	
MSI	Midline superior incisor
AIF	Anterior margin of incisive foramen
AIZ	Anterior inferior zygomatic
PM	Point of greatest curvature on the posterior margin of the malar process
ASA	Anterior superior alveoli
PIF	Posterior incisive foramen
PSA	Posterior superior alveoli
AIA	Anterior inferior auditory bulla
PZA	Point of greatest curvature along posterior edge of zygomatic process of temporal bone
OAS	Occipital-auditory-sphenoid junction
OA	Occipital-auditory junction
MPP	Medial palatal-pterygoid junction
LNS	Anteriormost point along lateral zygomatic-frontal suture.
NAS	Nasion (Midline)
IOS	Intersection of frontal suture with orbital rim
MS	Superior margin of suture of temporal and zygomatic processes of zygomatic arch.
FTP	Frontal-temporal-parietal junction
BRG	Bregma (Midline)
LAM	Lambda (Midline)
MST	Point along occipomastoid suture
TYM	Superoposterior extremity of tympanic ring
Mandibular landmarks	
MPM	Anterior edge of alveolar process where first molar hits alveolus at the midline
IAR	Intersection of molar alveolar rim and base of coronoid process
ACC	Anterior edge of the coalescence of curve of masseteric ridge with post-symphyseal rugged area
IDP	Superior-most point on incisor alveolar rim at midline (at bone-tooth junctions)
PCP	Apex of coronoid process
ANG	Tip of mandibular angle
AMC	Anterior midline point on condyle
PMC	Posterior midline point on condyle
IDI	Inferior-most point on incisor alveolar rim at midline (at bone-tooth junction)
SIR	Superior-most point on inferior border of mandibular ramus
AMF	Anterior edge of the mental foramen
ARF	Anterior edge of the ramal fossa foramen. If two foramina, use lower foramen
ART	Apex of retromolar trigone
ABC	Anterior base of coronoid process at midline
AMF	Anterior edge of mandibular foramen
MAC	Most concave point on subcondylar incisive

Abbreviation	Description
MN	Mandibular notch

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Table 3.

Means and standard deviations for cranial, mandibular and postcranial measures (mm).^{a,b}

Mean and standard deviation																<i>t</i> -test p-values					
C	M	W	C*M	C*W	W*M	C-M	C-W	M-W	C-C*M	M-C*M	C-C*W	W-C*W	M-W*M	W-W*M							
Postcrania																					
Humerus	11.0±0.5	10.8±0.2	11.2±0.3	11.7±0.4	11.7±0.4	11.6±0.3	0.008	0.066	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001						
Ulna	12.6±0.4	12.5±0.2	12.7±0.3	14.0±0.4	13.7±0.4	13.8±0.3	0.417	0.133	0.004	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001						
Femur	13.7±0.5	14.3±0.3	14.4±0.5	15.4±0.4	15.0±0.6	15.6±0.5	<0.001	<0.001	0.606	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001						
Fibula	14.0±0.4	14.0±0.2	14.4±0.3	15.4±0.4	15.2±0.4	15.3±0.4	0.311	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001						
Cranium																					
NAS-BRG	6.4±0.18	6.3±0.14	6.6±0.22	6.3±0.17	6.5±0.19	6.8±0.2	0.106	0.661	0.766	0.414	0.334	0.115	0.045	0.024	0.010						
BRG-LAM	3.6±0.36	3.6±0.25	4.1±0.2	4.1±0.2	4.4±0.25	4.1±0.23	0.997	0.874	0.939	0.722	0.830	0.690	0.435	0.405	0.252						
MSI-NAS	7.1±0.27	7.7±0.14	7.4±0.26	7.9±0.22	7.8±0.25	8.1±0.28	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001						
MSI-LNS	5.7±0.21	6±0.12	6.1±0.2	6.3±0.23	6.4±0.24	6.6±0.21	<0.001	<0.001	<0.001	<0.001	0.001	0.024	0.038	0.090	0.125						
MSI-AIF	2.2±0.09	2.5±0.08	2.4±0.09	2.4±0.07	2.3±0.08	2.6±0.09	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001						
AIF-PIF	5±0.22	5.4±0.09	5.2±0.14	5.8±0.15	5.7±0.13	5.9±0.16	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001						
AIF-AIZ	3.8±0.22	3.7±0.11	4.1±0.16	4.1±0.19	4.3±0.17	4.3±0.17	0.067	0.115	0.125	0.087	0.056	0.060	0.111	0.171	0.205						
AIZ-PM	2.1±0.12	2.2±0.07	2.4±0.12	2.4±0.13	2.4±0.11	2.5±0.15	0.015	0.018	0.018	0.026	0.043	0.087	0.086	0.142	0.244						
AIZ-ASA	2.9±0.21	3.3±0.09	3.4±0.17	3.4±0.17	3.4±0.14	3.5±0.18	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001						
PM-PZA	6.4±0.19	6.5±0.13	6.4±0.14	6.9±0.17	6.8±0.15	6.8±0.17	0.211	0.171	0.197	0.232	0.289	0.235	0.200	0.248	0.319						
PM-IOS	3.5±0.15	3.4±0.09	3.5±0.13	3.7±0.11	3.6±0.09	3.6±0.14	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001						
ASA-PSA	4±0.13	3.7±0.09	3.8±0.09	4.2±0.12	4.2±0.11	4.1±0.1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001						
ASA-PIF	1.7±0.08	1.9±0.08	1.8±0.06	1.9±0.06	1.9±0.09	2±0.07	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001						
PSA-MPP	1.2±0.12	0.9±0.09	1.1±0.07	1±0.12	1.1±0.08	1±0.09	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001						
PSA-FTP	5.5±0.17	5.8±0.09	6±0.18	5.9±0.12	6±0.12	6.2±0.15	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001						
PSA-PZA	4.5±0.15	4.6±0.08	4.5±0.12	4.9±0.13	4.8±0.12	4.9±0.14	0.014	0.021	0.026	0.042	0.047	0.042	0.043	0.055	0.059						
AIA-OA	3.4±0.1	3.4±0.09	3.7±0.08	3.6±0.09	3.7±0.08	3.7±0.1	0.417	0.515	0.816	0.836	0.932	0.907	0.770	0.559	0.479						
AIA-OAS	2.9±0.09	3±0.05	3.1±0.08	3±0.08	3.1±0.09	3.1±0.07	<0.001	<0.001	<0.001	<0.001	0.003	0.009	0.012	0.049	0.092						
AIA-PZA	3.1±0.13	3.1±0.08	3.2±0.12	3.4±0.09	3.4±0.1	3.4±0.12	0.202	0.116	0.063	0.026	0.009	0.003	0.002	0.001	0.001						
PZA-LAM	8±0.19	7.6±0.16	8.3±0.16	8.1±0.14	8.4±0.18	8.3±0.19	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001						
PZA-BRG	7.1±0.15	6.6±0.11	7±0.14	6.9±0.11	7.1±0.12	7±0.15	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001						

Mean and standard deviation															<i>t</i> -test p-values					
	C	M	W	C*M	C*W	W*M	C-M	C-W	M-W	C-C*M	M-C*M	C-C*W	W-C*W	M-W*M	W-W*M					
PZA-FTP	4.7±0.2	4.8±0.15	5.1±0.14	5±0.14	5±0.14	5.1±0.18	0.521	0.313	0.177	0.138	0.073	0.049	0.024	0.012	0.011					
PZA-MS	4.5±0.18	4.9±0.2	4.8±0.2	5.1±0.19	5±0.14	5.1±0.2	<0.001	<0.001	<0.001	<0.001	0.001	0.003	0.021	0.076	0.235					
OAS-OA	2.9±0.18	2.9±0.12	2.9±0.14	3±0.15	3±0.12	2.9±0.14	0.037	0.037	0.046	0.055	0.050	0.027	0.028	0.033	0.016					
LNS-IOS	0.8±0.12	1±0.1	0.8±0.1	1.1±0.14	0.9±0.11	0.9±0.11	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001					
NAS-FTP	5.1±0.18	4.7±0.15	4.9±0.13	5.1±0.14	5.1±0.12	5.1±0.13	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001					
MS-FTP	5.2±0.25	5.4±0.12	5.6±0.23	5.7±0.17	5.5±0.16	5.8±0.19	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.002	0.006					
FTP-BRG	3.9±0.16	3.5±0.12	3.7±0.19	3.3±0.09	3.7±0.09	3.6±0.11	<0.001	<0.001	<0.001	<0.001	0.001	0.018	0.116	0.342	0.759					
LAM-MST	5.7±0.16	5.5±0.09	6.1±0.14	5.8±0.12	5.9±0.1	6±0.13	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001					
MST-TYM	1.6±0.13	1.5±0.08	1.6±0.12	1.6±0.09	1.6±0.1	1.6±0.1	<0.001	0.026	0.129	0.525	0.934	0.930	0.531	0.264	0.138					
AIF-ASA	3.9±0.24	4.4±0.1	4.3±0.15	4.7±0.17	4.6±0.12	4.8±0.15	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001					
PIF-MPP	2.3±0.17	2.3±0.16	2.4±0.12	2.4±0.12	2.5±0.13	2.6±0.12	0.793	0.939	0.908	0.938	0.872	0.714	0.511	0.567	0.606					
MPP-AIA	5.7±0.33	5.5±0.18	5.7±0.21	6.1±0.26	5.9±0.22	6±0.21	0.049	0.036	0.023	0.014	0.006	0.002	0.001	<0.001	<0.001					
MPP-OAS	5.2±0.3	5.1±0.18	5.5±0.17	5.7±0.22	5.6±0.2	5.6±0.18	0.086	0.067	0.052	0.046	0.020	0.010	0.008	0.004	0.003					
LNS-AIF	5.5±0.25	5.4±0.1	5.8±0.17	5.9±0.23	6±0.18	6.1±0.17	0.437	0.314	0.229	0.103	0.032	0.017	0.009	0.003	0.001					
NAS-IOS	2.3±0.1	2.4±0.14	2.4±0.11	2.3±0.12	2.3±0.1	2.4±0.09	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.004	0.013					
IOS-MS	4.6±0.26	4.1±0.24	4.3±0.24	4.6±0.18	4.5±0.15	4.3±0.18	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.003	0.005					
MST-PZA	5.5±0.22	5.4±0.18	5.6±0.16	5.9±0.14	5.8±0.19	5.9±0.17	0.851	0.888	0.937	0.829	0.742	0.421	0.490	0.550	0.496					
GM	3.7±0.11	3.7±0.05	3.8±0.08	3.9±0.08	3.9±0.07	4.0±0.09	0.417	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001					
Mandible																				
MPM-IAR	2.1±0.15	2.1±0.13	2.1±0.15	2.1±0.14	2.2±0.14	2.2±0.13	0.025	0.961	0.020	0.793	0.033	0.531	0.558	0.001	0.420					
MPM-ART	5.3±0.14	5.6±0.14	5.9±0.22	6.1±0.28	6.1±0.19	6.1±0.21	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.037	<0.001	0.033					
MPM-AMF	1.2±0.11	2±0.13	1.8±0.14	1.6±0.11	1.6±0.13	1.8±0.17	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.475					
IAR-ABC	1.7±0.17	1.8±0.1	1.9±0.17	2±0.16	1.9±0.17	2±0.15	<0.001	<0.001	0.333	<0.001	<0.001	<0.001	0.179	0.001	0.051					
IAR-ART	1.9±0.1	2±0.18	2±0.12	2.1±0.12	2±0.15	2.2±0.11	0.029	0.009	0.898	<0.001	0.002	0.009	0.780	<0.001	<0.001					
ABC-PCP	1.6±0.15	2.2±0.12	2.3±0.15	2.3±0.2	2.2±0.19	2.3±0.16	<0.001	<0.001	0.021	<0.001	0.075	<0.001	0.476	0.002	0.393					
PCP-MN	0.8±0.08	1.1±0.08	1.1±0.08	1.1±0.1	1.1±0.1	1.1±0.08	<0.001	<0.001	0.158	<0.001	0.979	<0.001	0.027	0.239	0.010					
AMC-MN	1.4±0.13	1.4±0.16	1.8±0.19	1.8±0.14	1.9±0.12	1.7±0.16	0.490	<0.001	<0.001	<0.001	<0.001	<0.001	0.252	<0.001	0.042					
AMC-PMC	1.5±0.12	1.8±0.16	1.5±0.13	1.5±0.14	1.4±0.11	1.7±0.14	<0.001	0.277	<0.001	0.085	<0.001	0.497	0.077	0.281	<0.001					
PMC-MAC	2.2±0.12	2.5±0.12	2.8±0.18	2.9±0.17	2.8±0.26	3.1±0.15	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.163	<0.001	<0.001					
MN-AMF	1.9±0.1	2.1±0.09	2.1±0.12	2.2±0.11	2.2±0.1	2.2±0.11	<0.001	<0.001	0.059	<0.001	<0.001	<0.001	0.090	<0.001	<0.001					

	Mean and standard deviation						t-test p-values								
	C	M	W	C*M	C*W	W*M	C-M	C-W	M-W	C-C*M	M-C*M	C-C*W	W-C*W	M-W*M	W-W*M
MN-MAC	0.8±0.1	1.1±0.08	1.1±0.11	1.1±0.13	1±0.12	1.2±0.08	<0.001	<0.001	0.067	<0.001	0.718	<0.001	0.008	<0.001	<0.001
MAC-ANG	2±0.13	2.3±0.17	2.3±0.15	2.2±0.15	2.3±0.19	2.4±0.15	<0.001	<0.001	0.696	<0.001	0.515	<0.001	0.473	0.029	0.056
ANG-ARF	3±0.16	3.4±0.13	3.6±0.21	3.6±0.2	3.4±0.21	3.9±0.16	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	<0.001	<0.001
ARF-SIR	1.1±0.19	1.4±0.17	1±0.18	1.5±0.18	1.1±0.3	1.3±0.2	<0.001	0.018	<0.001	<0.001	<0.001	0.918	0.066	0.287	<0.001
SIR-ACC	2.7±0.17	2.3±0.18	2.5±0.19	2.5±0.19	2.7±0.29	2.4±0.16	<0.001	<0.001	0.019	<0.001	0.015	0.188	<0.001	0.573	0.055
ACC-IDI	2.9±0.14	3.1±0.13	3.6±0.12	3.2±0.15	3.4±0.13	3.5±0.11	<0.001	<0.001	<0.001	<0.001	0.021	<0.001	<0.001	<0.001	0.015
IDI-IDP	1.9±0.1	2.4±0.06	2.2±0.09	2.4±0.1	2.2±0.09	2.4±0.07	<0.001	<0.001	<0.001	<0.001	0.030	<0.001	0.720	0.015	<0.001
IDP-AMF	2.6±0.13	2.6±0.16	2.7±0.13	2.9±0.14	2.8±0.16	2.9±0.19	0.056	<0.001	0.029	<0.001	<0.001	<0.001	0.024	<0.001	<0.001
ACC-AMF	2±0.12	2.6±0.14	2.7±0.16	2.5±0.16	2.5±0.17	2.8±0.18	<0.001	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.018
SIR-AMF	3.9±0.27	4.3±0.24	4.5±0.29	4.1±0.26	4.6±0.37	4.4±0.33	<0.001	<0.001	<0.001	0.033	0.010	<0.001	0.650	0.166	0.057
GM	1.8±0.05	2.1±0.03	2.2±0.06	2.2±0.07	2.1±0.06	2.3±0.05	<0.001	<0.001	0.339	<0.001	<0.001	<0.001	0.401	<0.001	<0.001

^aResults of *t*-tests comparing means for cranial and mandibular measurements and limb lengths (postcrania) among parents, among hybrids, and between hybrids and their respective parents. Bolded values are significant at $p = 0.05$ and underlined at $p = 0.001$.

^bAbbreviations for strain: W refers to WSB, C refers to CAS and M refers to CZE, hybrids are designated as combinations of the parents with an * (e.g. C*W refers to the F1 hybrid of WSB and CAS). Comparisons between strains show strains and hybrids tested separated by a dash (-; e.g. C-C*W refers to a comparison between the parent, CAS, and the F1 hybrid CAS-WSB). Abbreviations for landmarks can be found in Table 2.

Table 4.

A summary table with percentage of averaged distances falling into each category.^a

	Cranium	Mandible	Postcranial
CASxCZE			
Intermediate	18	43	0
Larger, non-significant	79	57	100
Smaller, non-significant	3	0	0
Transgressive (larger)	58	52	100
Transgressive (smaller)	5	0	0
Heterosis	82	76	100
Dysgenesis	11	5	0
CASxWSB			
Intermediate	34	38	0
Larger, non-significant	63	57	100
Smaller, non-significant	3	5	0
Transgressive (larger)	55	14	100
Transgressive (smaller)	5	0	0
Heterosis	89	90	100
Dysgenesis	8	0	0
CZExWSB			
Intermediate	18	33	0
Larger, non-significant	82	62	100
Smaller, non-significant	0	5	0
Transgressive (larger)	58	43	100
Transgressive (smaller)	5	0	0
Heterosis	89	81	100
Dysgenesis	0	10	0

^aHere, “transgressive” refers to when hybrids are significantly larger or smaller than both parent strains, “heterosis” and “dysgenesis” refer to measures when hybrids are larger or smaller than the 95% CI of the MPV, respectively. This table also indicates when hybrids are intermediate to both parents and larger or smaller than both (regardless of significance).