samples (from 2- and 10-m depth) for DIC, Alk, and δ13C at Station 5, located 26 km southeast of the island of Bermuda (32°10′N, 64°30′W) (27). In 1989, the Joint Global Ocean Flux Study (JGOS) program was established at a station 56 km further southeast (31°50′N, 64°10′W) (38). The CDRG program extended their measurements to this new site, and the BCSR started to measure DIC there throughout the water column (24). Because differences between Station 5 and BATS have been found to be small (38), and because the DIC data from the two laboratories show no systematic differences (change in DIC = –0.01–0.20 m s⁻¹ N = 78 measurements), the data from the two sites and two laboratories are combined here into a single time series.

The reduced isotopic ratio, δ13C, is defined as

\[ \delta^{13}C = \left( \frac{^{13}C}{^{12}C} \right)_{sample} \left( \frac{^{12}C}{^{13}C} \right)_{standard} \times 1000 \]

where \( \left( ^{13}C/^{12}C \right)_{sample} \) is the sample, and \( \left( ^{13}C/^{12}C \right)_{standard} \) is the Pee Dee belemnite standard.

We computed the oceanic pCO₂ from the observed temperature, salinity, Alk, DIC, and nutrients using a threedimensional circulation model (39) and the dissociation constants of Mehrbach et al. (40) as refitted by Dickson and Millero (41). This choice is based on laboratory studies (42) and the resulting good agreement with direct observations of pCO₂ near Bermuda (43).

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References and Notes

3. This trend is smaller than that reported by Bates (41) for 1988 to 1998, mainly because of smaller growth rates in the 1980s.
8. Net community production refers to the net transfer between inorganic and organic carbon pools due to photosynthesis and to oxidation of organic matter, and therefore is equal to net primary production minus community respiration.
9. Materials and methods are available as supporting material on Science Online.
10. Because the time-series sites are located in the broad recirculation region of the subtropical North Atlantic, the geostrophic current means are from the north- east side of the sites, and indicate a mean current of about 0.05 m s⁻¹ (44), although substantially higher velocities are associated with the passage of eddies. Based on the observed mean horizontal gradient near Bermuda of about 1.1 x 10⁻³ mol kg⁻¹ m⁻¹ (29), our model-derived transport estimates give horizontal velocities of between –0.01 and –0.20 m s⁻¹, with a long-term mean of –0.07 m s⁻¹ (negative velocity indicates southward flow).
11. The uncertainties of the fluxes have been established with a Monte Carlo technique (45). The diagnostic model was run 1000 times, with randomly selected sets of initial values within a predetermined range for each parameter based on the parameter’s perceived uncertainty (29). The flux uncertainties listed in the text denote the 1-sigma uncertainty computed from the results of these 1000 realizations.
12. Because our diagnostic model analyses cannot uniquely determine the underlying mechanisms, other factors, such as variations in mesoscale eddy dynamics, or dust deposition and its possible impact on N₂ fixation, could be responsible for our positive correlation of net community production with winter mixed-layer depth anomalies.
15. The atmospheric CO₂ inversion results have been provided by P. Bousquet and P. Peylin and are based on the anomalous CO₂ fluxes for the entire North Atlantic region as estimated in their global study (4).
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Control of Facial Muscle Development by MyoR and Capsulin

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Members of the MyoD family of basic helix-loop-helix (bHLH) transcription factors control the formation of all skeletal muscles in vertebrates, but little is known of the molecules or mechanisms that confer unique identities to different types of skeletal muscles. MyoR and capsulin are related bHLH transcription factors expressed in specific facial muscle precursors. We show that specific facial muscles are missing in mice lacking both MyoR and capsulin, reflecting the absence of MyoD family gene expression and ablation of the corresponding myogenic lineages. These findings identify MyoR and capsulin as unique transcription factors for the development of specific head muscles.

The myogenic bHLH transcription factors—MyoD, Myf5, myogenin, and MRF4—control vertebrate skeletal muscle development (1). MyoD and Myf5 act redundantly as myoblast specification genes (2), whereas myogenin is required for myoblast differentiation (3, 4). MyoD and MRF4 also play redundant roles in muscle differentiation (5). Although these myogenic genes control a developmental program shared by all skeletal muscles, there is evidence that muscles in the head and trunk differ with respect to the early steps in myogenic lineage specification (6). For example, in Myf5⁻/⁻, Pax-3⁻/⁻ double-mutant mice, the trunk musculature is eliminated, but head muscles are unaffected (7). The developmental control genes responsible for head muscle formation are unknown.

All skeletal muscle posterior to the head is derived from paraxial mesoderm that becomes segmented into somites (1, 8). In contrast, head muscles are derived from multiple cell lineages, including prechordal mesoderm anterior to the first somite and paraxial mesodermal precursors that migrate into the branchial arches (9–11). MyoR/muscinul and capsulin/Pod-1/epicardin are related bHLH proteins transiently expressed in migratory paraxial mesodermal cells in the branchial arches that appear to represent precursors of the muscles of mastication (12–18).

A null mutation in the mouse capsulin gene results in neonatal lethality due to pulmonary hypoplasia, but no overt skeletal muscle abnormalities (18, 19). We combined mutations in MyoR and capsulin to see if there were shared activities or effects not revealed with either single-gene deletion. The MyoR gene was targeted in embryonic stem (ES) cells by homologous recombination (fig. S1A). ES cell clones...
harboring the mutant allele were used to generate chimeric mice, which transmitted the mutation through the germ line (fig. S1B). Breeding of mice heterozygous for the mutant MyoR allele yielded homozygous mutants at predicted Mendelian ratios with no obvious abnormalities.

Mice homozygous for the MyoR and capsulin null mutations were obtained by breeding heterozygous mutant mice. Double mutants were born alive, but, like capsulin−/− mice (18, 19), they died within minutes after birth. Histological examination revealed a complete absence of the major muscles of mastication, including the masseter, medial and lateral pterygoids, and temporalis muscles in the majority of double-mutant embryos (Fig. 1, A to E). In their place was connective tissue. In a subset of double mutants, atrophic pterygoid myofibers persisted unilaterally. The missing muscles in MyoR−/−capsulin−/− double-mutant mice are derived from the first branchial arch and represent a distinct group of muscles that function in mastication (8). Other first arch–derived muscles, such as the anterior digastric and mylohyoid, which do not function in mastication, were present in the double mutants [Fig. 1, A to D (and 20)]. Trunk muscles of double mutants were also indistinguishable from those of wild-type animals (20). Head muscle defects were not observed in MyoR−/−capsulin−/− or in MyoR−/−capsulin−/− embryos. These findings demonstrated that MyoR and capsulin redundantly controlled the formation of a specific subset of first arch–derived facial skeletal muscles and that a single copy of either gene is sufficient to support normal muscle development.

MyoR−/−capsulin−/− double mutants also displayed cleft palate (Fig. 1C). Other branchial arch–derived skeletal elements were normal in double mutants [Fig. 1 and (20)]. In addition, the visceral organs of MyoR−/−capsulin−/− double-mutant neo-

**Fig. 1.** Deficiency of head skeletal muscle and diaphragmatic hernia in MyoR−/−capsulin−/− neonates. Coronal sections from MyoR−/−capsulin−/− (A and B) and MyoR−/−capsulin−/− (C and D); (B and D) show a higher magnification of (A and C), respectively. Note the cleft palate (p) in (C). gl, glands; m, mandible; ma, masseter muscle; p, palate; pt, pterygoid muscles; t, tongue; te, temporalis muscle. Asterisks in (C) denote missing muscles. In (B), the glands abut the masseter, whereas in (D), the masseter is missing and the glands abut the mandible. (E) Diagrams of muscle groups missing from the double mutant. (F) Sagittal section of a MyoR−/−capsulin−/− with diaphragmatic hernia. Arrowheads mark the boundaries of the diaphragmatic defect. Arrow marks the diaphragm. d, diaphragm; g, gut; l, liver; p, pancreas. Scale bars, 200 μm.

**Fig. 2.** Expression of capsulin-lacZ allele. Expression of lacZ in capsulin−/− mice at E7.5 (A) and E8.0 (B and C), as indicated by arrowheads. (C) A coronal section through the embryo in (B). (D to F) LacZ staining is present within the first and second branchial arches in MyoR−/−capsulin−/−. (G to I) Staining gradually disappears from the first arch of MyoR−/−capsulin−/−. (J and K) Sections of the first arch of embryos in (F) and (I), respectively. 1, branchial arch 1; 2, branchial arch 2. Arrowheads in (J) and (K) indicate the muscle developing around the fifth cranial nerve. LacZ-positive cells are missing from this region of the double mutant. Scale bars: (A, B, and C), 200 μm; (D to I), 500 μm; (J and K), 100 μm.
capsulin expression was detected in the first branchial arches of wild-type embryos by in situ hybridization. Capsulin was detected at E8.5 in the first arch (ba1). Expression of Myf5 and MyoR overlapped that of capsulin at later stages. (B) Myf5 expression was detected in the first branchial arches of MyoR<sup>−/−</sup>capsulin<sup>−/−</sup>, designated wild type, but not in MyoR<sup>−/−</sup>capsulin<sup>−/−</sup> embryos. The middle panels show higher magnification of the upper panels. ba1, first branchial arch; ba2, second branchial arch; hc, hypoglossal cord; m, Myotome. (C) Myf5, myogenin, and MyoD expression was detected in wild-type and MyoR<sup>−/−</sup>capsulin<sup>−/−</sup> embryos at E15.5. The masseter muscle (ma) is completely absent in MyoR<sup>−/−</sup>capsulin<sup>−/−</sup> mutants (designated ma<sup>a</sup>). Arrow indicates cleft palate. Scale bars: 200 μm for (A and B), 1 mm for (C).

The head muscle deficiency in MyoR<sup>−/−</sup>capsulin<sup>−/−</sup> double mutants could arise from a defect in specification, migration, or proliferation of the affected myogenic lineages; a block in myoblast differentiation; or an increase in apoptosis. To determine the basis for this muscle deficit and to pinpoint the time of its onset, we compared the expression of a lacZ reporter integrated into the targeted capsulin allele in staged embryos of the different genotypes. From embryonic day 7.5 (E7.5) to E8.0, when mesodermal precursors of first arch muscle cells initially appear subjacent to the metencephalon (10), lacZ staining was localized to this muscle precursor population in embryos of the different genotypes (Fig. 2, A to C). At E9.5, these lacZ-positive cells could be seen migrating into the newly formed branchial arches of wild-type and double-mutant embryos (Fig. 2, D and G). By E10.5, a swathe of lacZ-positive cells extended into the first and second branchial arches. This myogenic precursor pool appeared to migrate properly, but there were noticeably fewer of these lacZ-positive cells in the first branchial arch of the double mutants (Fig. 2, E and H). In contrast, other embryonic sites of lacZ expression showed similar staining in embryos of the different genotypes. At E11.5, lacZ expression was observed in presumptive myoblasts within the first and second branchial arches of normal embryos (Fig. 2F). These lacZ-positive cells within the first branchial arch surrounded the fifth cranial nerve (Fig. 2J). There was a dramatic reduction of lacZ staining in the myogenic cores of the first branchial arches of double mutants at E11.5 (Fig. 2, I and K), suggesting that the capsulin-expressing myogenic lineage of the first branchial arch was specifically affected in these mutant embryos.

The striking lack of specific facial skeletal muscles in MyoR<sup>−/−</sup>capsulin<sup>−/−</sup> double mutants was reminiscent of the phenotype associated with the combined absence of Myf5 and MyoD, except that the latter phenotype affects all skeletal muscles (2). To define the temporal sequence of expression of these genes and to determine whether myogenic bHLH genes were expressed in the affected muscle lineage of the double mutant, we performed in situ hybridization with adjacent sections of staged embryos between E8.5 and 15.5. Capsulin transcripts were detected in mesodermal precursors of the first arch at E8.5 (Fig. 3A). At this stage, Myf5, MyoD, and MyoR ex-
pression was undetectable. There has been some disagreement about the timing of expression of Myf5 and MyoD in the branchial arches, depending on the method of detection, but the earliest reported expression of these genes in this region is E9.25 and E9.5, respectively (22–24). By E9.5, Myf5 and capsulin were expressed in the same cell population within the first branchial arch, and by E10.5, Myf5, capsulin, and MyoR were coexpressed in these cells of wild-type embryos (Fig. 3A). In contrast, Myf5 was not expressed in first branchial arch precursors of MyoR−/−capsulin−/− double mutants at E9.5 or E11.5 (Fig. 3B). There was also no evidence for expression of Myf5, MyoD, or myogenin at E15.5 in the region of affected facial muscles (Fig. 3C), whereas these genes were expressed in other developing head and trunk muscles.

To determine the fate of first arch muscle precursors that failed to activate expression of Myf5 and MyoD, we performed TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling) on histological sections of double-mutant embryos at E10.5, when cells marked by expression of capsulin-lacZ were disappearing. As shown in Fig. 4, TUNEL-positive cells were observed among the lacZ-positive muscle precursors of double mutants at E9.5 or E11.5 (Fig. 3B). We conclude that these cells, which fail to initiate the normal program for muscle development in the double mutant, undergo apoptosis with resulting ablation of muscles of mastication. Similar observations have been made in muscle precursor cells in the limb buds of mice lacking MyoD and myf5 (25).

The absence of specific head muscle cells, as well as markers of the corresponding myogenic lineages, in MyoR−/−capsulin−/− mutants resembles the effect of MyoD−/−Myf5−/− double mutations on all skeletal muscles (2) and is distinct from the phenotype of Myf5−/−Pax3−/− mutants, which exhibit a specific deficiency of trunc muscle cells (7). This phenotype also differs from that of myogenin mutant mice, in which myoblasts express myogenic bHLH genes, but are unable to differentiate (3, 4). These findings demonstrate that MyoR and capsulin redundantly regulate an initial step in the specification of a specific subset of facial skeletal muscle lineages and that, in the absence of these factors, myogenic bHLH genes are not switched on, and cells from these lineages undergo programmed cell death. There may also be a modest effect on migration of precursors, as is seen in Lbx1 mutant mice (21). MyoR and capsulin act as transcriptional repressors in transfection assays (12, 20). Whether they act to repress an inhibitor of myogenogenesis or have a transcriptional-activating function during development of facial muscle remains to be determined. The phenotype of MyoR−/−capsulin−/− mutant mice reveals a previously unanticipated complexity in the development of head skeletal muscles, and these findings identify MyoR and capsulin as unique transcriptional regulators for the development of specific head muscles.

References and Notes
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Genetic Structure of Human Populations

We studied human population structure using genotypes at 377 autosomal microsatellite loci in 1056 individuals from 52 populations. Within-population differences among individuals account for 93 to 95% of genetic variation; differences among major groups constitute only 3 to 5%. Nevertheless, without using prior information about the origins of individuals, we identified six main genetic clusters, five of which correspond to major geographic regions, and subclusters that often correspond to individual populations. General agreement of genetic and predefined populations suggests that self-reported ancestry can facilitate assessments of epidemiological risks but does not obviate the need to use genetic information in genetic association studies.

Most studies of human variation begin by sampling from predefined “populations.” These populations are usually defined on the basis of culture or geography and might not reflect underlying genetic relationships (1). Because knowledge about genetic structure of modern human populations can aid in inference of human evolutionary history, we used the HGDP-CEPH Human Genome Diversity Cell Line Panel (2, 3) to test the correspondence of predefined groups with those inferred from individual multilocus genotypes (supporting online text).

The average proportion of genetic differences between individuals from different human populations only slightly exceeds that between unrelated individuals from a single population (4–9). That is, the within-population component of genetic variation, estimated here as 93 to 95% (Table 1), accounts for most of human genetic diversity. Perhaps as a result of differences in sampling schemes (10), our estimate is higher than previous estimates from studies of comparable geographic coverage (4–6, 9), one of which also used microsatellite markers (6). This overall similarity of human populations is also evident in the genetically widespread nature of most alleles (Fig. S1). Of 4199 alleles present more than once in the sample, 46.7% appeared in all major regions represented: Africa, Europe, the Middle East, Central/