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Evolutionary mutant models for human disease

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Although induced mutations in traditional laboratory animals have been valuable as models for human diseases, they have some important limitations. Here, we propose a complementary approach to discover genes and mechanisms that might contribute to human disorders: the analysis of evolutionary mutant models in which adaptive phenotypes mimic maladaptive human diseases. If the type and mode of action of mutations favored by natural selection in wild populations are similar to those that contribute to human diseases, then studies in evolutionary mutant models have the potential to identify novel genetic factors and gene-by-environment interactions that affect human health and underlie human disease.

Mutant models for human disease: use and limitations

Among the major biological insights of the late 20th century was the discovery that a large portion of the genes and mechanisms that direct embryonic development are broadly conserved among metazoans [1]. This discovery galvanized the use of traditional animal models (from flies to mice) for the study of traits and phenomena relevant to human health. Although most organisms are not recognized as 'model species', the same conserved genetic features are likely to operate in an array of animal species that exhibit extensive phenotypic diversity and, therefore, non-model species can provide important additional insights into human health and disease. Here, we propose that the investigation of evolutionary mutant models (see Glossary) can enhance our basic understanding of the genetic and developmental basis for human diseases.

Mutations induced in forward genetic screens in laboratory animals provide highly useful models of human phenotypic variation and disease that have led to previously unsuspected insights into human pathology [2–4]. This approach, however, is not without limitations. Mutagenesis screens often identify phenotypes that are more severe and/or have earlier onset than the human diseases they model. Because researchers often identify the most visible mutations in laboratory screens, induced mutations are predominantly in the coding regions of genes and lead to the severe attenuation or complete abrogation of gene function. Furthermore, because forward genetic screens typically recover mutant animals with defects at the earliest developmental stage in which the gene provides an essential function, they often mask developmental pleiotropy. As a result, phenotype-driven mutation screens using chemical mutagens in zebrafish, *Drosophila melanogaster* and *Caenorhabditis elegans* usually yield early defects that preclude the examination of phenotypes at the later life stages that are relevant to many human diseases.

In contrast to mutants recovered in laboratory screens, many naturally occurring mutations in humans that either cause a disease or increase disease susceptibility under specific environmental conditions are caused by alterations in the regulatory regions of genes [5–10]. Because multiple promoters, enhancers and silencers regulate the timing, abundance and cell-specific localization of gene expression, alterations in *cis*-regulatory elements can result in normal gene expression early in development but failed regulation of the activity of a gene in older individuals. For example, lactase persistence is a condition that results in the

Glossary

Association mapping: a method for mapping traits without a pedigree by scoring variation segregating within natural populations. Association mapping is based on the concept of linkage, such that individuals that inherit a functional mutation should also inherit alleles at nearby loci. Because population-level genealogies are much deeper than pedigrees and, hence, have experienced meioses over many generations, association mapping can usually map traits to a finer interval than pedigree mapping.
Conserved noncoding (CNC) sequence: a DNA sequence that does not contribute to the mature transcript of a gene but is highly conserved across distantly related taxa. CNCs can contain tens to hundreds of base pairs and many are considered to be putative regulatory regions of gene activity.
eQTL: a quantitative trait locus that affects gene expression. The search for eQTLs uses transcriptional profiling as a phenotype that can be mapped to assess how segregating allelic variation effects transcriptional regulation.
Evolutionary mutant models: an extant assemblage of related organisms in which certain variants (populations or species) express a phenotype that mimics the clinical features of a human disease. The gene pool and genomic organization of evolutionary mutant models result from many generations of natural selection and genetic drift.
Genetic architecture: a set of genetic loci and their interactions that account for an expressed phenotype.
Linkage disequilibrium: the non-random association of alleles at two or more loci. The rate of recombination, rate of mutation, genetic drift, non-random mating and population structure can all affect the degree of linkage disequilibrium.
Quantitative trait loci (QTL) analysis: a search for loci that affect variation of a quantitative trait. It is a statistical analysis of genotype-by-phenotype correlations using pedigrees of known structure, in which individuals are genotyped at marker loci spread across the genome and then the inheritance of each marker is compared to that of a phenotype. A QTL is localized to a particular region of the genome when the segregation of marker loci within that region matches the segregation of phenotypic variation.

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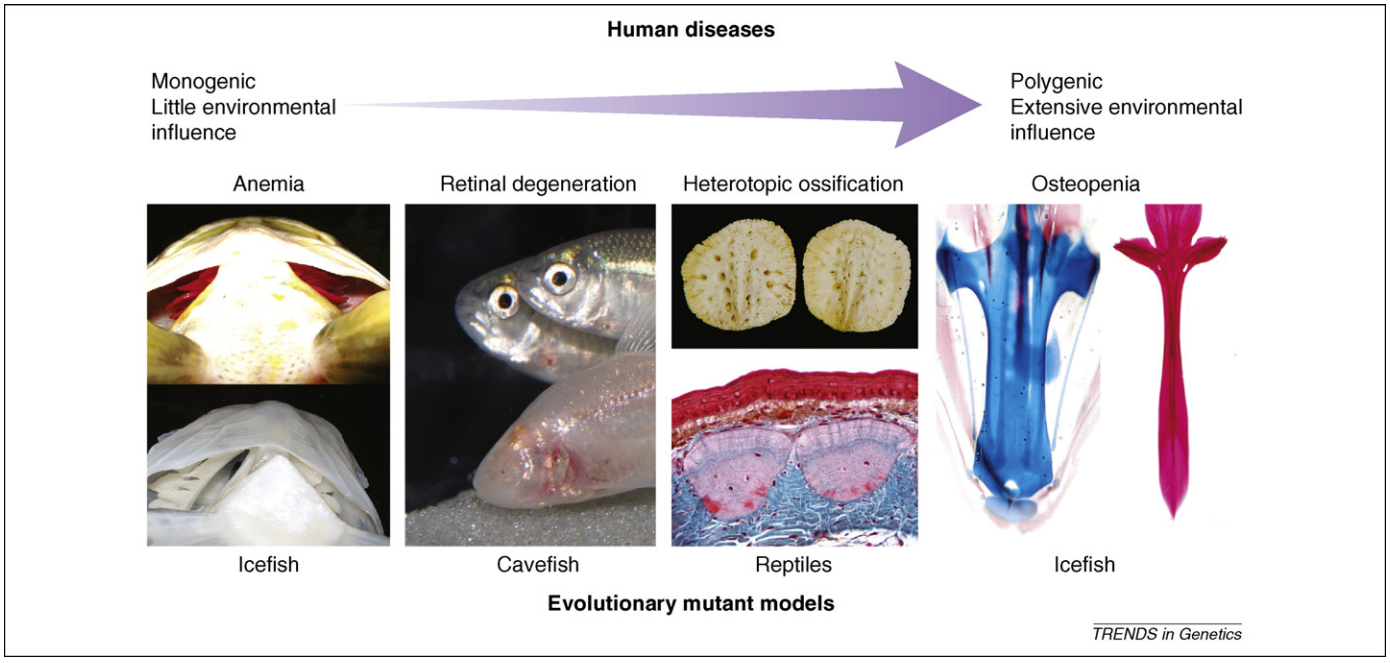

Figure 1. Examples of evolutionary mutant models for human disease. Evolutionary mutant models have the potential to reveal novel insight into the genetic basis of an array of different types of human diseases, from simple to complex. An example of a natural system that models a simple human disease is anemia in icefish. Note that the icefish (lower panel) lacks red blood cells (as seen in the gills) compared with the closely related rockcod (upper panel). Evolutionary mutants can also model complex human diseases including osteopenia in icefish. Note that the base of the icefish neurocranium is cartilaginous (blue, left panel) compared with the closely related rockcod, which has a mineralized neurocranium (red, right panel). Evolutionary mutants will also be useful for diseases in the middle of the continuum that are affected by alleles of major effect, but of which expression is complex and variable. Diseases of this type include retinal degeneration and heterotopic ossification, which are modeled by blind cavefish and reptile osteoderms, respectively. Cavefish image reproduced with permission from R. Borowsky. Osteoderm image courtesy of M. Vickaryous.

sustained ability of an individual to digest lactose through adulthood [11]. Most human babies produce intestinal lactase, an enzyme that digests the milk sugar lactose. In many human populations, however, lactase production decreases during teenage years, blocking the digestion of lactose and enabling it to be fermented by bacteria in the colon, which causes abdominal pain. The ability to maintain lactase production is inherited in an autosomal dominant fashion and is associated with non-coding variation upstream of the lactase (*LCT*) gene that increases its expression, resulting in prolonged ability to digest lactose. Individuals lacking these variants gradually lose *LCT* activity and lack the ability to digest lactose as adults. Such regulatory mutations, when they occur in nature, are exposed to selection and can therefore be studied in evolutionary mutant models.

Human diseases lie along a continuum from 'simple' to 'complex'. On one end of the spectrum are monogenic disease conditions that exhibit simple Mendelian inheritance and express little phenotypic variation in individuals with the same allele, such as albinism or cystic fibrosis. On the opposite end of the spectrum are polygenic diseases, such as cancers or heart disease, the expression and severity of which are highly variable and depend on both the genotype of an individual at multiple loci and the environment. In the middle are disease traits that are affected by alleles of major effect, but in which expression is variable and affected by both genetic background and the environment, for example, cleft palate. Although induced mutant models have been extremely useful in studying simple diseases, they have been less successful in deducing the etiology and pathophysiology of complex diseases. We propose that evolutionary mutant models can compliment traditional induced mutant models to understand the genetic basis of both simple and complex human diseases (Figure 1).

Evolutionary mutants and the diseases they model

Occasionally, evolution by natural selection or genetic drift has resulted in populations with evolved phenotypes that mimic human disease, but are nevertheless adapted to their environment. Here, we discuss selected examples of evolutionary mutant models that can inform our understanding of human disease. Although our focus is largely on fish models, other metazoan systems undoubtedly provide additional evolutionary mutant models for human disease phenotypes and the principles discussed apply across many species.

Antarctic icefish as a model of anemia

Anemias are diseases with diminished numbers of red blood cells, resulting in decreased oxygen delivery to tissues. The 16 'white-blooded' species of the Antarctic icefish family (Notothenioidei: Channichthyidae) are unique among vertebrates because they do not express the developmental program for erythrocyte formation. Icefish neither make hemoglobin nor do they produce erythrocytes and, therefore, they model harmful human blood diseases, including anemias, hemoglobinopathies and thalassemias. In a genome-wide scan of hematopoietic tissues comparing white-blooded icefish with red-blooded relatives, transcriptome-based representational difference analysis (cDNA RDA; [12]) revealed that the novel gene *bloodthirsty* (*bty*) is expressed in the red-blooded relative but not in

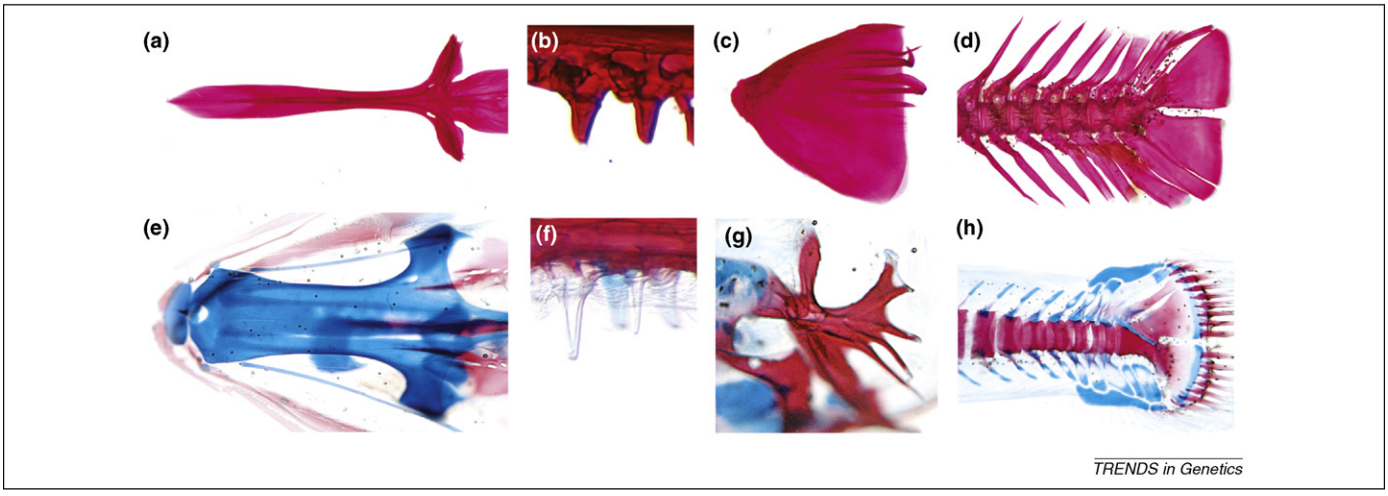

Figure 2. Osteopenia in Antarctic notothenioids. The evolutionary history of certain Antarctic notothenioid fish species has led to changes in the musculoskeletal system to increase buoyancy. Among other adaptations, several species have evolved bone loss and now possess greatly reduced bony skeletons. These evolutionary adaptations model human diseases of decreased bone mineralization including osteopenia. An example of an osteopenic notothenioid species is *Pseudochaenichthys georgianus* **(e-h)**, which, compared with the closely related but robustly mineralized species, *Notothenia rossii* **(a-d)**, shows reduced levels of bone mineralization in the base of the skull (a,e), oral teeth (b,f), opercular bone (c,g) and caudal skeleton. Images show the staining of cartilage with alcian blue and bone with alizarin red in juvenile fish.

the white-blooded icefish [13]. The antisense knockdown of *bty* in zebrafish and the rescue of its expression by mRNA injection confirmed that *bty* has a crucial role in erythrocyte development [14]. The apparent human ortholog of *bty* belongs to the tripartite motif (*TRIM*) gene family, which has many members that encode E3 ubiquitin ligases (H. W. Detrich, unpublished). Thus, Bty is likely to target a repressor of proerythroblast differentiation for degradation by the proteosome, thereby inducing terminal erythroid differentiation. These studies demonstrate that evolved genetic changes in icefish can provide a model for human anemias.

Antarctic icefish as a model of osteopenia

Osteopenia is a reduction in bone mineral density (BMD) that affects ~34 million American women and 12 million American men. It can lead to osteoporosis, a disease characterized by low bone mass, bone deterioration, bone fragility, increased susceptibility to fracture and slow healing of bone fracture [15]. More than half of Americans over 50 years old have osteoporosis (http://www.nof.org). Because of their unique evolutionary history, certain lineages of Antarctic fish provide striking evolutionary mutant models of reduced bone density diseases. Specifically, in several notothenioid species, natural selection has favored structural changes in the musculoskeletal system to increase buoyancy, including the replacement of densely mineralized bone by connective tissue and the decreased ossification of the skeleton [16]. Figure 2 shows the dramatic difference in mineralization of the skeletons of an osteopenic juvenile icefish and a closely related robustly ossified species of comparable developmental stage. The identification of genetic factors that underlie these evolved differences has the potential to lead to a better understanding of the mechanisms that regulate bone density.

Blind cavefish as a model of retinal degeneration

Many cave-dwelling species evolve degenerated lenses and retinas, similarly to humans with retinal degeneration diseases [17,18]. The Mexican tetra (*Astyanax mexicanus*)

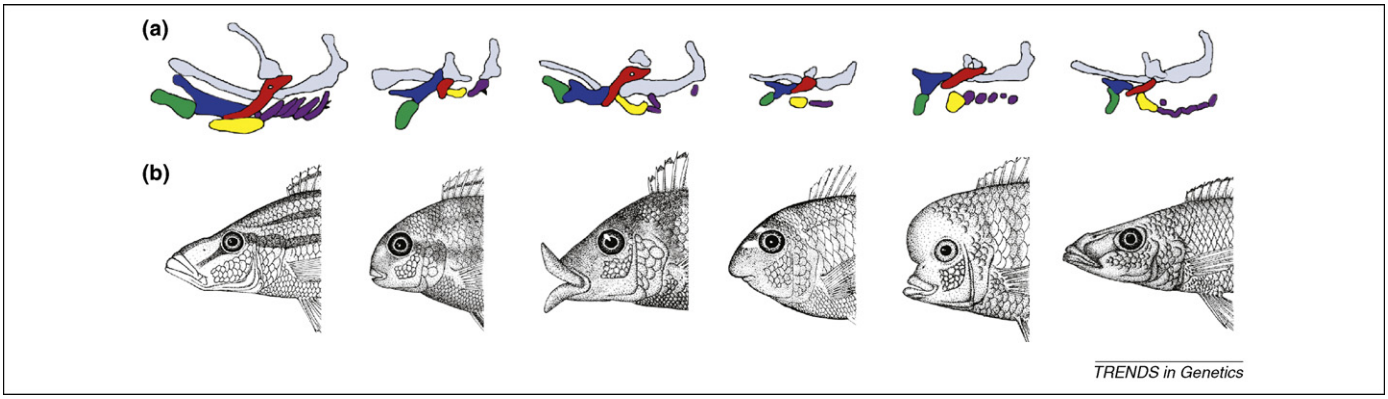

Figure 3. Induced versus evolutionary mutant models. (a) Chemically induced zebrafish craniofacial mutants (wild-type configuration is to the left). Shaded shapes represent different pharyngeal cartilage elements depicted in the lateral view with the rostral–caudal axis running from left to right. Induced laboratory mutants have been useful for deducing the factors that pattern the embryonic craniofacial skeleton, including (left to right following the wild-type configuration) *low/tfap2a, fla/pold1, bab/rerea, dak/ext2* and *her* [25]. Note differences in the number, size and orientation of elements. Color code: gray, skull cartilages; green, lower jaw; blue, upper jaw; red, hyosympletic cartilage; yellow, ceratohyal cartilage; purple, posterior gill cartilages. Reproduced with permission of the Company of Biologists. **(b)** A series of evolutionary mutants illustrates the striking difference in craniofacial shape among closely related cichlid species. Here, natural selection has screened random mutations for differences in adult feeding morphology. These evolutionary mutants model both normal and clinical variation in human craniofacial architecture. Cichlid illustrations were drawn by R.C.A.

has both surface populations with eyes and cave populations without eyes, providing an ideal opportunity to study the genetic basis of evolved eye loss. Relative to surface forms, expression of *pax6,* which when mutated in human and mouse causes malformation of iris and lens [19,20], was shown to be downregulated in the developing optic field of blind cavefish [21]. Furthermore, the experimental overexpression of two upstream regulators of eye development, *sonic hedgehog* (*shh) a* and *shhb*, in surface fish resulted in the downregulation of *pax6* and reduction of the optic cup, which indicates that the hedgehog signaling pathway is important in the evolution of cavefish eye degeneration [22]. It was, therefore, surprising that a recent mapping study showed that the causative genetic variants for eye loss in blind cavefish are not linked to *shha, shhb* or *pax6* [18], implicating other factors in the evolution of this phenotype. At least four to six unlinked loci are involved in cavefish eye loss [23] and different cavefish populations show varying degrees of eye degeneration, underscoring the complexity of this trait [24]. The molecular genetic factors that actually cause the evolution of eye loss in blind cavefish have yet to be identified, but their subsequent localization might provide novel candidate genes and pathways for human retinal degeneration. The regressive evolution of eye loss was probably a gradual process during the transition from a surface to cave environment and the genetic changes that occurred over evolutionary time might mimic changes in gene regulation that occur over developmental time in genetically complex degenerative eye diseases of old age.

East African cichlid fish as a model of craniofacial disease

Craniofacial diseases involve distortions in the construction of the face and jaw. An emerging challenge for developmental biologists is to learn the molecular determinants of craniofacial shape, and the challenge for physicians is to convert that knowledge into therapies and preventative practices. Closely related cichlid fishes from lakes in the East African Rift Valley have undergone extensive evolutionary modifications of their oral jaws and faces, providing an array of evolutionary mutant models for medically important human craniofacial variation.

Just as chemically induced zebrafish mutants have been used to study craniofacial patterning [25], natural populations of cichlids can be used to study the development of craniofacial shape (Figure 3). Diversity in cichlid tooth size, shape and number, for example, provides a useful model for human hyperdontia (i.e. Apert syndrome), hypodontia (i.e. Crouzon syndrome) and macrodontia (i.e. hemifacial hyperplasia) [26]. Some cichlids eat the scales of other fish and are genetically specialized to feed from the left or right side of the prey [27]. Many of these scale-eating species have evolved skeletal and dental asymmetries similar in presentation to individuals with hemifacial microsomia and hemifacial hyperplasia [28]. Both in the adaptive situation in the fish and the disease state in humans, the ventral derivatives of the first and second pharyngeal arches are affected and the asymmetries have a directional bias. Because few laboratory mutants are characterized by craniodental asymmetries (but see Ref. [29]), analyses of laterality in scale-eating cichlids have the potential to reveal novel factors involved in regulating craniofacial symmetry. Compared with laboratory-based mutagenesis in a few distantly related model organisms (e.g. zebrafish and mouse), a comparative approach to jaw development in a group of closely related, but phenotypically divergent, cichlid species provides a robust opportunity to estimate the number, type and mode of action of genes involved in jaw morphogenesis. Continued work on cichlids will further our understanding of the genetic changes and the gene-by-environment interactions that affect evolutionary adaptation and provide new targets for the prediction and treatment of complex human craniofacial disorders.

Reptiles as models of heterotopic ossification

Heterotopic ossification (HO) refers to the formation of bone in soft tissues. In humans, this condition is a frequent complication of injuries to the central nervous system, hip surgeries and burns [30] and some people might be genetically predisposed to HO after injury [31]. There are also several rare inherited forms of HO, including fibrodysplasia ossificans progressiva (FOP), progressive osseous heteroplasia (POH) and Albright's hereditary osteodystrophy [32]. Several recent studies implicate altered bone morpho-

Box 1. Does natural selection target 'disease genes'?

The argument that analyses in evolutionary mutant models might lead to a better understanding of the genetic nature of human disease traits relies on the assumption that the same genes are implicated in both human diseases and adaptive phenotypes. Work in several evolutionary mutant models indicates that evolutionary divergence sometimes involves genes previously implicated in human diseases. For example, various populations of blind cavefish (*A. mexicanus*) have evolved pigment loss by the recurrent fixation of mutations in the coding region of ocular and cutaneous albinism-2 (*OCA2*), the most commonly mutated gene in cases of human albinism [47]. The evolution of reduced pigmentation has also occurred in different populations of threespine stickleback and a recent study has shown that *cis*-regulatory changes in the *KIT ligand* gene (*kitl/KITLG*) are responsible for differences in pigmentation in both stickleback and human populations [51]. In domestic dogs, a candidate gene approach was used to search for genes that might be involved in the diversification of skull shapes [52,53]. One such study focused on Treacher Collins-Franceschetti syndrome 1 (*TCOF1*), the gene that causes Treacher Collins Syndrome (TCS; Online Mendelian Inheritance in Man® [OMIM®] number 154500), an autosomal dominant disease that affects craniofacial development and leads to variable facial anomalies, including malformations of the outer ears, cleft palate and mandibular hypoplasia [54]. In a survey of dog breeds, distinct haplotypes of the canine homolog of *TCOF1* were shown to be associated with differences in head shape, particularly in the length and width of the skull [52]. In a similar study, allelic variation in insulin-like growth factor-1 (*IGF1*), a gene linked to congenital growth defects in humans [55], was shown to be responsible for differences in the size of dogs [56]. Finally, turtle shells form through intramembranous ossification of the dermis [41] and, whereas the turtle carapace is distinct from osteoderms, it represents another elaboration of the dermal skeleton through heterotopic bone formation. Similarly to patients with FOP, BMP signaling in the intercostal dermis has been implicated in this type of heterotopic ossification [41]. These studies show that genes known to have a role in human diseases are also involved in evolutionary change, which supports the hypothesis that evolutionary mutants are appropriate models for the study of human disease.

genetic protein (BMP) signaling as a primary cause of FOP [33,34], and decreased expression of the α subunit of the stimulatory G protein (GNAS) of adenylyl cyclase has been linked to POH [35]. Despite this progress, the pathophysiology of acquired and inherited forms of HO remains unclear [30]. In HO, the process of bone formation itself seems to be normal, but the temporal and spatial patterns of skeletogenesis are misregulated. Osteoderm development in squamate (scaled) reptiles could provide a model to better understand the onset and progression of these diseases. Osteoderms are secondary dermal ossifications that fortify the integument and serve to protect, camouflage and ornament organisms that possess them. Among tetrapods, osteoderms have evolved independently in reptiles (i.e. gecko lizards), amphibians (i.e. horned frogs) and mammals (i.e. armadillos) [36]. Although the specific mode of osteoderm ossification varies, all osteoderms develop from within connective tissues that possess latent skeletogenic potential [37]. In reptiles, osteoderms form when connective tissues transform into bone without cellular de-differentiation, osteoblasts or a periosteum, similarly to FOP [38]. Whether the extensive variation in osteoderm size, shape and number found among closely related lizard species involves the same genes implicated in human HO pathologies or as yet undiscovered genes remains to be seen (Box 1). What is clear is that these evolutionary novelties mimic HO in both anatomy and developmental sequence. In humans, HO is progressive and degenerative and is subject to environmental and genetic factors, whereas in reptiles, it is restricted and adaptive.

The nature of genetic variation in the wild and the tools to study it

The molecular genetic nature of phenotypic variation in evolutionary mutant models differs considerably from that of mutants induced in the laboratory. Laboratory mutagenesis screens use a mutagen to 'break' genes, which perturbs normal development and leads to large phenotypic effects that investigators easily identify and sort. By contrast, natural selection and genetic drift seal the fate of novel mutations in the wild, often leading to the accumulation of many alleles with small to moderate effect on phenotypes. To be maintained over evolutionary time, new natural genetic variation must also properly integrate into established developmental and physiological systems. Evolutionary mutant models are, therefore, more likely than induced mutations to harbor genetic variation that affects the regulation of tissue-specific, post-embryonic and/or adult phenotypes. Thus, differences between evolutionary mutant models and mutants induced in the laboratory include: (i) the degree of exhibited phenotypic variation; (ii) the numbers of genes involved in producing this variation; (iii) the magnitude of mutational effects; (iv) the parts of the gene affected by mutations; and (v) the age of onset of allelic effects. Tools for the genetic analysis of evolutionary mutant models must be able to accommodate these differences (Box 2).

Choosing evolutionary models: practical considerations

Although the main consideration for choosing an evolutionary mutant model will be its phenotypic similarity to a

Box 2. Tools for the analysis of evolutionary mutant models

The genetic analysis of traits in evolutionary mutant models is similar to that used for induced mutations in that both require the recovery of offspring from defined matings, which can best occur in controlled crosses in the laboratory. However, in contrast to genetic mapping of a single induced mutation with high penetrance, complex traits in natural populations are mapped using QTL analysis. In the former, the correlation between phenotype (mutant versus wild type) and genetic markers is usually perfect and the precision of genetic mapping is limited only by the number of available recombination events. In QTL mapping, the association becomes explicitly statistical because many different combinations of factors (genetic and environmental) can lead to similar phenotypes. Moreover, for quantitative traits, the expectation of uniform expressivity of mutations across genetic and environmental variation does not hold, which is similar to many human diseases. For example, the rate of diabetes in Pima Indians living in Mexico (7%) is less than one-fifth of that found in genotypically similar Pima Indians living in the United States (38%) [57], a dramatic effect of environment on phenotypic expression of a single genotype. Thus, QTL mapping is not simply a way of mapping traits in the absence of a mutagen, rather it allows for the characterization of gene function within a variable genetic and environmental background.

Despite the strengths of pedigree mapping in evolutionary mutant models, some organisms cannot be readily crossed in the laboratory (e.g. birds, reptiles and large mammals). Association mapping in natural populations provides an additional way to correlate phenotypic variation with specific genomic regions. This technique relies on the extent of linkage disequilibrium, which should be much smaller in natural populations, with their numerous generations of recombination, than in laboratory crosses [58]. The decreasing costs of massively parallel sequencing, as well as a plethora of new genotyping technologies, is making association and population genomic mapping quite feasible even for non-model systems [59].

Evolutionary mutant models can often involve differences in gene expression (as opposed to complete loss-of-function mutations identified in the laboratory), especially those mutations that affect regulatory regions of specific genes. Therefore, in addition to the genetic mapping of causal alleles, identification of variation in levels of transcription or translation through mRNA or protein abundance, respectively, can provide hints pointing to evolving pathways or be used as confirmatory evidence for one of a set of candidates in a QTL region. In systems in which QTL mapping is not possible, expression analysis might also be used to identify candidate genes and pathways for evolved phenotypic differences. Methods to detect variation in transcription can be performed at the level of individual genes (e.g. qPCR) or on a genome-wide scale [60]. A particularly useful synthesis of technologies combines whole genome transcriptional profiling with genetic mapping to identify expression QTL (eQTL) [61].

Once candidate genes for the phenotypic trait in the evolutionary mutant model have been mapped using laboratory crosses or association analysis and verified by differences in expression, one ultimately wants to identify the particular nucleotide changes responsible for observed evolved phenotypic change. If sequencing rules out causative genetic change in the coding regions of evolutionary mutant models, thereby implicating regulatory elements, then a potentially fruitful approach is to search for conserved noncoding (CNC) sequences near the identified gene of interest through multiple alignment analysis [62]. Definitive proof of genotype–phenotype relationships, however, must come from manipulative studies that show that specific nucleotide substitutions cause observed phenotypic change. This last 'proof of principle' step will not be possible in many evolutionary mutant models but, in some cases, the same approaches used in laboratory models will be useful for confirming the genetic basis for evolutionary mutant phenotypes, including allelic introgression, gene knockdown, phenocopies and phenotype rescue experiments.

given human disease, several practical considerations come into play. First, the most useful evolutionary models will most likely be those that have evolved rapidly. Rapid evolution provides the opportunity to make genetic crosses among variant populations to dissect the identity, number and mode of action of loci affecting complex traits. Reproductive barriers in rapidly evolving systems are often prezygotic, which makes it possible to obtain fertile, interspecific hybrids by *in vitro* fertilization.

Second, the magnitude of phenotypic divergence among closely related species is important because it affects the statistical power for the detection of quantitative trait loci (QTL) in line-cross experiments [39]. Among vertebrates, bony fishes are exemplary evolutionary models [40]. No other vertebrate class exhibits comparable levels of phenotypic diversity or rates of speciation and, as described earlier, investigators can use this diversity to screen a wide spectrum of traits.

A third important criterion is an understanding of the biogeography and phylogeny of an evolutionary model system. Studying organismal design in the context of a well-resolved phylogeny facilitates insight into the patterns and tempo of evolutionary change, which in turn informs an understanding of how phenotypes evolve in response to environmental change. Are evolutionary shifts incremental or has adaptation involved saltatory events? Do distinct traits evolve independently or as units? The answer to these and related questions can help us deduce the underlying genetic complexity of, and linkage between, complex phenotypes. The turtle shell and the bat wing, for example, evolved without obvious evolutionary intermediates, indicating that the appearance of these morphological novelties was sudden and might have involved simple molecular genetic changes and heterotopic shifts of preexisting regulatory networks [41–43]. In cichlids, analyses of the evolutionary diversification of dentition have revealed a link between tooth size, shape and number, such that selection on one results in coordinated changes in the others [26]. Interestingly, genetic changes that control the periodic patterning of the odontogenic program seem to be simple [44,45]. These findings are novel and relevant to craniodental development in general. Phylogenetic reconstructions can also reveal the direction of evolutionary change and whether different taxa have similar traits owing to independent evolutionary events or shared ancestry.

Finally, systems that have repeated independent convergence on a phenotype are particularly useful because they offer the opportunity to study the genetic basis of complex phenotypes in replicate natural experiments, which models the repeated occurrence of complex human diseases in different populations (e.g. cleft palate; [46]). Furthermore, the genetic architecture and propensity of a trait for molecular convergence will provide insight into the complexity of the regulatory networks that underlie its development. For example, although *oca2* is implicated in the convergent evolution of albinism in different cavefish populations [47] (Box 1), multiple genetic factors are likely to be involved in convergent loss of cavefish eyes [48]. The *oca2* gene seems to be a 'large' target for evolutionary change in pigmentation and one might predict that the evolution of albinism in other cave-dwelling species (e.g. amphibians, shrimp, spiders) might also be a result of mutations in *oca2*. That a similarly 'large' target has not been found for eye loss underscores the anatomical, developmental and genetic complexity of this organ. In a similar way, the many factors implicated in human cleft palate highlight the complexity of palatal development (for a review, see Ref. [49]). Compared with albinism in blind cavefish, genetic analyses of eye loss in different cavefish populations have the potential to reveal a multitude of targets for human degenerative eye disease.

Concluding remarks and future perspectives

Human diseases lie along a continuum from 'simple' to 'complex' and the use of evolutionary mutant models should extend to both ends of this spectrum. For example, whereas adaptive traits that phenocopy simple human disease states might be polygenic (e.g. eye and lens degeneration in humans and cavefish), knowledge of these additional loci can provide insight into the biology and expressivity of the human disease trait, pinpoint candidate genes and pathways that can be examined functionally in more traditional laboratory models, and provide potential targets for therapeutics. Studies of evolutionary mutant models also promise a much better understanding of the combined polygenic and environmental components of many complex disease traits including osteoporosis, heart disease or diabetes. Although the majority of examples discussed in this article are of more simple disease models, examples of evolutionary mutant models for complex diseases also exist and include hypertension in giraffes [50] and osteopenia and anemia in Antarctic notothenioid fish (discussed earlier). Examples of evolutionary mutant models for human diseases are few because this line of inquiry is in its infancy and the tools and resources for developmental genetic analyses of organisms in natural populations have only recently become available. Nevertheless, the studies highlighted here attest to the utility of this approach and, with subsequent technological advances, we expect that evolutionary mutant models will rapidly become a valuable tool for deciphering the interacting genetic and environmental risk factors of many human diseases.

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