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The genetic basis of modularity in the development and evolution of the vertebrate dentition

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The construction of organisms from units that develop under semi-autonomous genetic control (modules) has been proposed to be an important component of their ability to undergo adaptive phenotypic evolution. The organization of the vertebrate dentition as a system of repeated parts provides an opportunity to study the extent to which phenotypic modules, identified by their evolutionary independence from other such units, are related to modularity in the genetic control of development. The evolutionary history of vertebrates provides numerous examples of both correlated and independent evolution of groups of teeth. The dentition itself appears to be a module of the dermal exoskeleton, from which it has long been under independent genetic control. Region-specific tooth loss has been a common trend in vertebrate evolution. Novel deployment of teeth and reacquisition of lost teeth have also occurred, although less frequently. Tooth shape differences within the dentition may be discontinuous (referred to as heterodonty) or graded. The occurrence of homeotic changes in tooth shape provides evidence for the decoupling of tooth shape and location in the course of evolution. Potential mechanisms for region-specific evolutionary tooth loss are suggested by a number of mouse gene knockouts and human genetic dental anomalies, as well as a comparison between fully-developed and rudimentary teeth in the dentition of rodents. These mechanisms include loss of a tooth-type-specific initiation signal, alterations of the relative strength of inductive and inhibitory signals acting at the time of tooth initiation and the overall reduction in levels of proteins required for the development of all teeth. Ectopic expression of tooth initiation signals provides a potential mechanism for the novel deployment or reacquisition of teeth; a single instance is known of a gene whose ectopic expression in transgenic mice can lead to ectopic teeth. Differences in shape between incisor and molar teeth in the mouse have been proposed to be controlled by the region-specific expression of signalling molecules in the oral epithelium. These molecules induce the expression of transcription factors in the underlying jaw mesenchyme that may act as selectors of tooth type. It is speculated that shifts in the expression domains of the epithelial signalling molecules might be responsible for homeotic changes in tooth shape. The observation that these molecules are regionally restricted in the chicken, whose ancestors were not heterodont, suggests that mammalian heterodonty may have evolved through the use of patterning mechanisms already acting on skeletal elements of the jaws. In general, genetic and morphological approaches identify similar types of modules in the dentition, but the data are not yet sufficient to identify exact correspondences. It is speculated that modularity may be achieved by gene expression differences between teeth or by differences in the time of their development, causing mutations to have cumulative effects on later-developing teeth. The mammalian dentition, for which virtually all of the available developmental genetic data have been collected, represents a small subset of the dental diversity present in vertebrates as a whole. In particular, teleost fishes may have a much more extensive dentition. Extension of research on the genetic control of tooth development to this and other vertebrate groups has great potential to further the understanding of modularity in the dentition.

Keywords: dissociability; meristic systems; heterodonty; odontodes

1. INTRODUCTION

The discipline of evolutionary biology has traditionally attempted to identify mechanisms of phenotypic evolution in the form of external forces acting on organisms (Levinton 1988; Raff 1996; Kirschner & Gerhart 1998). Recent progress in understanding the developmental mechanisms by which the phenotype is specified by the genotype has led to an interest in intrinsic features of organisms that contribute to their evolvability—the ability to produce adaptive phenotypic variation (Levinton 1988; Wagner 1996; Wagner & Altenberg 1996; Raff 1996; Gerhart & Kirschner 1997; Kirschner & Gerhart 1998; Von Dassow & Munro 1999; Raff & Raff

2000). It has been suggested that one such feature is the organization of the phenotype into 'modules', units that are semi-autonomous in evolution and potentially so in function. The dissociability of these modules in evolution is believed to be facilitated by a corresponding modular organization of the genetic pathways controlling their development, such that pleiotropic effects of mutations in genes fall to a greater degree within modules than between them. As pointed out by Wagner (1996), such modules correspond to the homologues of comparative anatomy.

It is not surprising that body parts as distinct as brains and limbs are dissociable from each other in their evolution; such parts are built according to distinct structural plans. A greater challenge for explanation is posed by the dissociable evolution of different portions of meristic systems, i.e. those composed of multiple, similarly constructed parts. Such systems include the leaves of plants, segments of arthropods and hairs of mammals, with the individual elements considered to be serially or iteratively homologous (Bateson 1894; Riedl 1978; Wagner 1989). In order for some members of these series to evolve independently of others, there must exist at least some developmental and genetic individualization within the series (Wagner 1989). The extent to which this is the case has been a subject of inquiry dating back at least to Bateson (1892, 1894), who posed the question in terms of whether individual elements of a meristic series in one species had specific homologues in the series of another.

An ideal system for addressing the developmental genetic basis of modularity within meristic systems and the dissociable evolution of such modules is the vertebrate dentition. Because of the prominence of teeth in the fossil record and their accessibility for measurement in living forms, the pattern of vertebrate dental evolution has been documented in exquisite detail. As discussed below, numerous possibilities exist for groups of teeth-forming modules. In addition, the past few years have witnessed significant progress in identifying genes involved in the control of tooth development (reviewed by Peters & Balling 1999; Tucker & Sharpe 1999; Jernvall & Thesleff 2000*a*). The present work addresses the extent to which phenotypic modules within the dentition (identified by morphological differences and independent evolution from other modules) develop under independent genetic control. The main trends in the morphological evolution of the dentition are sketched, with an emphasis on dissociable changes among groups of teeth. Genetic evidence for the independent control of the development of groups of teeth is reviewed and used to speculate on the mechanisms by which dissociable evolutionary change has occurred. Finally, several approaches likely to generate important new empirical evidence on modularity and dissociation in the dentition are described.

2. MODULARITY IN THE DENTITION

(a) *Structure and development of teeth*

The teeth of jawed vertebrates consist of a main body of the calcified tissue dentine surrounding a pulp cavity rich in blood vessels and nerves (Peyer 1968). Dentine can usually be distinguished from bone by the presence in the former of tubules which enclose processes of the cells (odontoblasts, Ruch *et al*. 1995) that secrete the organic matrix. The crown surfaces of teeth are usually covered with a hypermineralized layer (enamel or enameloid). The organic matrix of enamel is produced by epithelially derived cells known as ameloblasts, while that of enameloid is produced by both ameloblasts and odontoblasts (Shellis 1978; Zeichner-David *et al*. 1995). While the mode of attachment of teeth to underlying skeletal structures varies extensively, in many cases this involves bony tissues derived from the tooth germ itself (Peyer 1968; Fink 1981; Lumsden 1988; Smith & Hall 1993; Ten Cate 1995).

Jawed vertebrate tooth development (most extensively characterized in mammals) involves both epithelial and mesenchymal tissues, which contribute to the final structure of the organ and undergo a series of reciprocal inductive interactions (Thesleff & Hurmerinta 1981). The first morphological sign of mammalian tooth development is a thickening of the oral epithelium to form a dental lamina (Butler 1956; Ruch 1995; Jernvall 1995). This epithelial structure then invaginates into the underlying mesenchyme to form a bud. Mesenchymal cells condense around this bud, eventually giving rise to the dental papilla, from which dentine and pulp develop, and the surrounding dental follicle, from which attachment tissues are derived (Lumsden 1988; Ten Cate 1995; Chai *et al*. 2000). Folding of the epithelial component gives rise to the enamel organ and the shape of the junction of the enamel organ and dental papilla mirrors the crown shape of the final tooth (Butler 1956).

(b) *The dentition as a module of the dermal exoskeleton*

It has long been noted that teeth share structural and developmental similarities with components of the calcified dermal exoskeleton of 'lower' vertebrates (reviewed by Ørvig 1977; Schaeffer 1977; Reif 1982; Smith & Hall 1993; Smith & Coates 1998, 2000). Such dermal skeletal structures include the scales ('dermal denticles') of chondrichthyan (cartilaginous) fishes, and superficial layers of dermal bones, scales, fin rays and fin spines of extant and fossil osteichthyan (bony), as well as fossil agnathan (jawless) fishes. The term 'odontode' has been applied to teeth and the tooth-like portions of these other structures (Ørvig 1977; Reif 1982). Identifying unambiguous criteria to differentiate teeth from other types of odontodes has proven difficult; location, function and developmental pattern have been suggested (Ørvig 1977; Reif 1982; Smith & Hall 1993; Smith & Coates 1998, 2000). In the latter case, development from an epithelial invagination rather than more superficial development is the primary consideration. Some odontodes may differ greatly in shape from true teeth, suggesting that the two systems are developmentally individualized and therefore form separate modules. An example is provided by relatively continuous sheets of fused odontodes on dermal bones, scales and fin spines of the primitive ray-finned bony fish *Polypterus* (Meinke 1982). In other cases, odontodes may be shaped like teeth, as is the case for the dermal denticles of chondrichthyans and spine-like structures on the scales of the primitive ray-finned fish *Polypterus* (Reif 1980, 1982; Meinke 1982). Despite such superficial similarities in shape between chondrichthyan dermal denticles and teeth, they

may undergo independent evolution. An extreme example is provided by stingrays (myliobatiforms). Most members of this group have elongated stinging spines attached to the tail, which are believed to be modified dermal denticles (Compagno 1999; Kemp 1999). Within this group, the teeth of myliobatids (eagle and cow-nose rays) have become modified into large flat-crowned crushing plates (Compagno 1999). In addition to dermal denticles covering the external surface of the body and true teeth on the margins of the upper and lower jaws, many chondrichthyans have denticles lining portions of the oropharyngeal cavity (Nelson 1970). Reif (1980, 1982) has shown that the dermal denticles, teeth and oropharyngeal denticles do not grade into each other morphologically and develop by different mechanisms, as well as at different times. As such, all three are likely to constitute separate modules of the dermal skeleton.

Bony fishes may possess teeth on virtually any of the bones lining the oropharyngeal cavity. In contrast to the situation with chondrichthyans, it is not clear which of these are true teeth and which are a different type of odontode (Ørvig 1977; Reif 1982; Smith & Coates 2000). For the purposes of this review, all such structures in the oropharyngeal cavity will be referred to as teeth. Odontodes outside the oropharyngeal cavity have been greatly reduced in extant bony fishes and are completely lacking from their derivatives, the tetrapods (Smith & Hall 1993; Smith & Coates 1998, 2000; Huysseune & Sire 1998). It has long been thought that teeth in the oropharyngeal cavity evolved by the encroachment of dermal denticles into this region (Smith & Hall 1993; Smith & Coates 1998, 2000). Initially, the oropharyngeal denticles would have been under similar or identical developmental genetic control, but would later have become individualized into separate modules (Smith & Coates 1998, 2000). This scenario was largely based on the presence of teeth and dermal denticles in chondrichthyans and the apparent earlier appearance of extraoral odontodes rather than teeth in the fossil record. New findings and interpretations of fossil vertebrates suggest that odontodes appeared virtually simultaneously in dermal armour and the oropharyngeal region (Smith & Coates 1998, 2000). These authors point out that it is possible that oropharyngeal denticles evolved first or that there never was a stage when oropharyngeal and dermal odontodes were controlled by the same developmental mechanism. Whichever is the case, it is at least clear that teeth and other types of odontodes have long represented individual modules of the dermal skeleton. Interestingly, there may be a few cases where odontodes outside the oropharyngeal cavity have arisen from the tooth developmental programme more recently; these will be considered below for the insight they contribute to mechanisms of the evolution of tooth location.

(c) *The distribution of teeth in jawed vertebrates*

The location of teeth and other forms of odontodes in chondrichthyans has been described above. It is generally assumed that the primitive condition of the dentition of osteichthyan fishes was the presence of small tooth plates covering the entire surface of the oropharyngeal cavity (Nelson 1969). Although this condition is not known in any fossil or recent taxon, the dentition may nevertheless be quite extensive in some groups (figure 1). When considered as a group, bony fishes may possess teeth in the oral cavity on the marginal dermal bones of the mandibular arch and any of the dermal bones forming the palatal surface (Gosline 1971). Teeth may also be found on toothplates (with bony bases presumed to be homologous to bone of attachment, Smith & Hall 1993) attached to endochondral bones of the palate (Arratia & Schultze 1991) or free in the skin between palatal bones (e.g. the primitive teleost *Elops*, Nybelin 1968). In the pharyngeal region, teeth are borne on bony toothplates or gill rakers (considered to be homologous, Nelson 1969). These structures may be closely associated with or fused to endochondral elements forming the gill arch skeleton or they may be free in the skin between them (Nelson 1969). Tooth plates or gill rakers may be present on the hyoid arch or any of the five gill arches and, in the latter case, on any of the elements arrayed from ventral to dorsal: median basibranchials, and paired hypobranchials, ceratobranchials, epibranchials and pharyngobranchials (Nelson 1968, 1969; Arratia & Schultze 1990). The Chinese paddlefish *Psephurus*, a primitive ray-finned fish, has many free denticles in the oral epithelium not associated with tooth plates (Grande & Bemis 1991). Additional tooth-bearing locations in bony fishes include elements of the shoulder girdle in the bowfin *Amia calva*, garfish (Lepisosteidae) and a number of fossil groups, the ventral surface of the median urohyal (which underlies the basibranchials) in fossil teleosts and the branchiostegal rays of *Amia* and *Elops* (Patterson 1977; Arratia & Schultze 1990). The stromateoid teleost fishes possess teeth in oesophageal sacs posterior to the fifth gill arch (Haedrich 1967).

Additional locations of potentially true teeth outside of the oropharyngeal cavity include the external surface of the opercle and interopercle (elements of the gill cover) of trichomycterid catfishes (Arratia 1990), on scutes covering the entire trunk of two families of armoured catfishes (see Sire & Huysseune 1996 and included references), on the external surface of most of the dermal skull bones of the teleost *Denticeps clupeoides* (Sire *et al*. 1998), and on the bills of xiphiid and istiophorid teleosts (swordfish, sailfish and marlin) (see Ørvig 1977; Huysseune & Sire (1998) and included references). These 'teeth' outside of the mouth cavity are generally considered to be odontodes (Ørvig 1977). However, as discussed in § 4, rather than being related to the odontodes of more primitive groups of fishes, there is evidence that they might represent the activation of an existing tooth developmental programme in extraoral locations.

As in the case of bony fishes, tetrapods may possess teeth on the jaw margins as well as bones of the palate (Carroll 1988; Smith & Coates 1998, 2000). Although pharyngeal teeth may have been present in some fossil amphibians, such teeth are absent in all extant tetrapods (Smith & Coates 2000).

(d) *Potential modules within the dentition*

Modularity at the lowest level of morphological organization of the dentition might apply to individual cusps within multicuspid teeth, such as mammalian molars. Jernvall (2000) and Jernvall & Jung (2000) argued that the crown pattern of mammalian molars develops

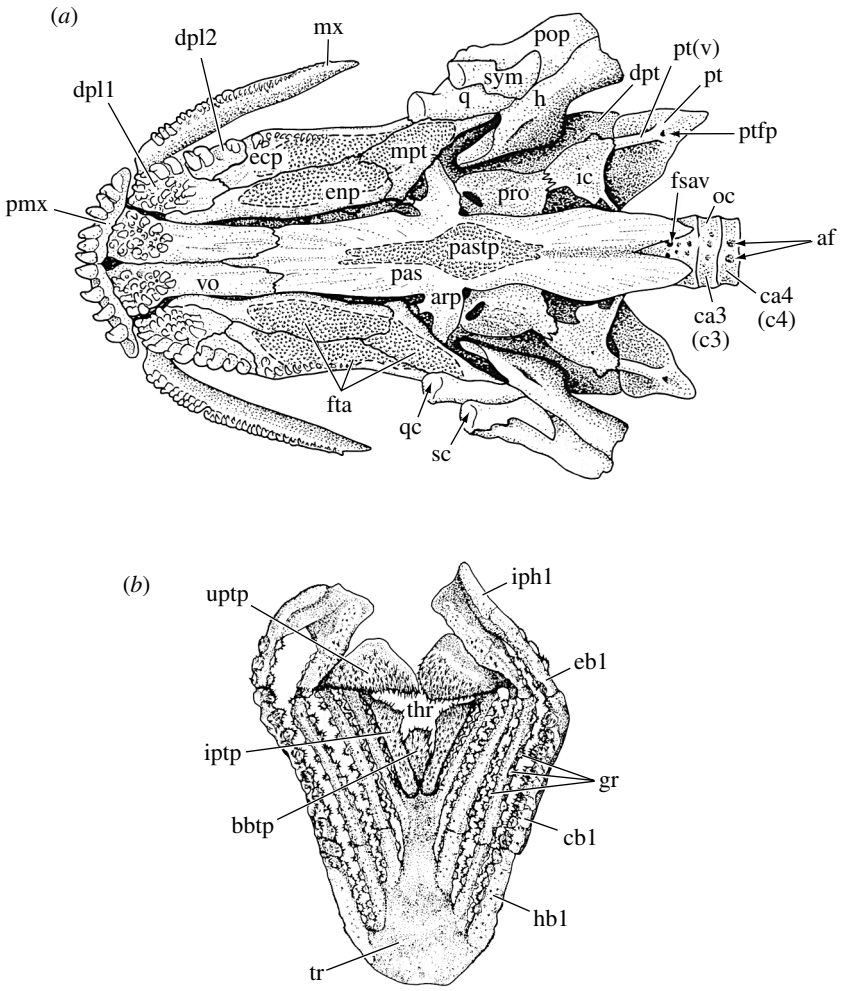

Figure 1. Dentition in the oral and pharyngeal cavities of the primitive ray-finned fish *Amia calva*. Reproduced from Grande & Bemis (1998) with permission from the Society of Vertebrate Paleontology. (*a*) Ventral view of jaw margin and palate. Teeth are present on premaxillae (pmx), maxillae (mx), dermopalatines (dpl1, dpl2), vomers (vo), ectopterygoids (ecp), endopterygoids (enp), metapterygoids (mpt) and the parasphenoid (pas). (*b*) Anterior view of pharyngeal arches. Toothed gill rakers are present on hypobranchials (hp), ceratobranchials (cb) and epibranchials (eb). Additional teeth are present on basibranchial element (bbtp), fifth ceratobranchials (lptp) and upper pharyngeal tooth plates (uptp).

through the repeated activation of a genetic pathway for making a cusp, with this pathway constituting a module. Given a cusp-making module, the question arises as to whether each cusp represents a different module, i.e. is individualized. The individuality of mammalian molar cusps is implied by a nomenclature that has been used to designate presumed homologous cusps between teeth within a species and between species (Osborn 1907; Hershkovitz 1968; Butler 1978*a*). Butler (1995) noted that cusps are relatively independent in variation and evolution and suggested that the tooth surface could be thought of as 'a mosaic of cuspal areas, each under specific genetic control'. Conversely, Jernvall (2000) and Jernvall & Jung (2000) pointed out that the genes known to be involved in cusp development appear to be the same for all cusps and proposed that crown patterns are determined sequentially as part of a 'patterning cascade'. In this model, the identity of a cusp is not specified by the expression of specific genes acting as a 'code'. Instead, crown patterns are encoded by parameters of a dynamic process, such as a reaction-diffusion mechanism, that activates the cusp module at specific locations and times during tooth development. Jernvall & Jung (2000) argued that despite the absence of genetic differences between specific cusps, these could still be homologous between species. It is worth noting that even if they exist, genes whose expression differs among cusps might be expected to represent a small fraction of all genes involved in cusp development, based on what is known about specification of vertebrate fore- and hindlimb identity (Ruvinsky & Gibson-Brown 2000).

In many vertebrates, tooth number is indeterminate and increases throughout life (Roberts 1967; Nakajima 1990; Berkovitz 2000). In such cases, it seems quite unlikely that each tooth is individualized. In some lineages, however, including mammals (Luckett 1993) and many characiform (Roberts 1967) and cypriniform (Nakajima 1990) fishes, tooth number is stable in adults and characteristic of a particular species. The nomenclature of mammalian teeth (Luckett 1993) implies that each individual tooth has a homologue in another species and therefore is also individualized in its developmental genetic

control. Early students of mammalian dental evolution believed that each tooth evolved its specific shape independently from that of other teeth as a result of natural selection (see references in Butler 1967). In contrast to this view, it has been argued that difficulties in identifying the homologies of teeth when their number varies within a species are an indication that each tooth is not individually encoded in the genome (Bateson 1892, 1894; Butler 1939, 1995). While genetic data (described below) exist for modular boundaries between teeth, they are not extensive enough to suggest each tooth as a separate module.

In most jawed vertebrates, teeth at particular locations within the dentition are replaced one or more times (Peyer 1968). In cases where replacement teeth develop in physical contact with an existing tooth, it is possible to designate tooth families consisting of a tooth and all of its successors (Reif 1982; Butler 1995; Huysseune & Sire 1998). Teeth of the same generation or of the same family therefore constitute potential modules within the dentition. Numerous species of characiform teleost fishes provide an example of greater resemblance between all teeth of one generation than between members of the same tooth family. In this group, the entire larval dentition is unicuspid, whereas subsequent generations of replacement teeth within a family have an increasing number of cusps (Roberts 1967). In mammals, 'milk molars', or deciduous premolars, are replaced by permanent premolars. In primitive perissodactyls (e.g. fossil horses), all permanent premolars exhibit characteristic differences from all milk molars (Butler 1952), raising the possibility of tooth generations as modules. During perissodactyl evolution, individual premolars have acquired the crown pattern of molars (molarization). Such molarization occurred sequentially at positions along the dentition and simultaneously affected the premolar and milk molar at a given position (Butler 1952). This pattern is suggestive of tooth families as modules, with members undergoing correlated change independent of other tooth families.

In lower vertebrates, teeth are generally named according to the bone to which they are attached and treated as homologous characters. This implies that teeth on separate bones form separate modules of the dentition. Indeed, tooth shape differences may correspond with bony sutures in a variety of vertebrates. In mammals, upper incisors are generally defined as teeth attached to the premaxillary bone, while the canine, premolars and molars are attached to the maxillary bone (Luckett 1993; Miles & Grigson 1990). Extinct placodont reptiles also possessed incisiform teeth on the premaxillae and molariform teeth on the maxillae and palatines (Carroll 1988) and the boundary between the premaxillary and maxillary bones corresponds to a boundary between multicuspid and blade-like teeth in the characiform fish *Landonia* (Roberts 1973). However, in all three of these groups, similar shape differences also exist among teeth attached to a single bone, the dentary. Sutures between bones need not correspond to shape differences among teeth. In many primitive bony fishes, denticle fields consisting of small teeth of similar shape extend across sutures; an example is provided by the ectopterygoid, endopterygoid and metapterygoid teeth of *Amia* (Grande & Bemis 1998; fig. 1). In addition, teeth on the ectopterygoid of piranhas, characteristically conical in other characiform fishes, resemble the flattened, blade-like teeth of the jaw margins (Roberts 1969).

The at least partial developmental independence of teeth from underlying bones or cartilage is illustrated by mutagenesis of laboratory animals as well as by patterns of evolution. As pointed out by Smith & Coates (1998) and Graveson *et al.* (1997), evidence for this independence is provided by several mutations in zebrafish in which the fifth ceratobranchial cartilage is lost while the teeth that normally become attached to it remain (Schilling *et al.* 1996). Evolutionary loss of fifth ceratobranchials in some eels has been accompanied by a shift of the tooth plates originally supported by them to the fourth ceratobranchials (Nelson 1969). According to Nelson (1969), the primitive condition for the bony fish oropharyngeal dentition is for tooth plates to be free in the skin. Only secondarily did these become supported by or fused with the endoskeletal elements of the branchial arches. Nelson (1969) suggested that unattached toothplates are variable in size, shape and position, but become more stable characters when associated with gill arch elements. This trend is interesting as a potential first step in the evolution of independent modules in the dentition, but the genetic basis of such an evolutionary event is completely unknown. In contrast to the above evidence for the independent evolution of teeth and bones, there is some evidence that teeth and bones may evolve together (Butler 1995). Differences in tooth size in two species of insectivore appear to be accompanied by changes in skull proportions (Butler 1941). In addition, tooth location may shift relative to the skull in the process of evolution; in general the shape of a tooth is correlated with its position in the skull (Butler 1941, 1995). For example, the broadest tooth in the mammalian maxilla is generally below the root of the zygomatic process, regardless of its position in the tooth series (Butler 1978*b*).

It is generally assumed that dental mesenchyme in all vertebrates is derived from the neural crest (Smith & Hall 1993), although this has only been directly demonstrated for mammals (Lumsden 1988; Imai *et al.* 1996; Chai *et al.* 2000) and amphibians (De Beer 1947; Chibon 1966). In contrast to this single germ layer of origin for tooth mesenchyme, the epithelial component of teeth may be derived from either ectoderm or endoderm. The enamel organs of mammalian teeth develop from ectoderm adjacent to endoderm (Imai *et al.* 1998), urodele amphibian enamel organs may be derived from ectoderm, endoderm or both (Adams 1924; De Beer 1947; Chibon 1970), and the enamel organs of fifth ceratobranchial teeth of the carp have been shown to be derived from endoderm (Edwards 1929). Although teeth derived from different germ layers might conceivably represent separate modules, the existence of enamel organs of mixed germ layer origin suggests that this may not be the case.

It is possible that modules within the dentition reflect patterning information within the three germ layers that is present well before the appearance of teeth. The time at which the pattern of the branchial arches and facial processes is specified and the germ layers which play a role in the process (ectoderm, neural crest, mesoderm and/or endoderm) have been studied extensively but remain controversial (for a review, see Hall 1999; Le

Douarin & Kalcheim 1999; see also Hunt *et al.* 1998; Trainor & Krumlauf 2000). Ectoderm, neural crest and mesoderm of the branchial arches undergo an extensive ventrad migration prior to occupying their final position (Noden 1991). In the case of neural-crest-derived ectomesenchyme, it has been shown that the rostrocaudal organization of premigratory neural crest along the neural tube is related to both rostrocaudal and dorsoventral organization of these cells once they reach the facial processes and pharyngeal arches (Hall 1999; Le Douarin & Kalcheim 1999). Interestingly, it has recently been found that elements of the facial and visceral skeleton may be compound structures formed from neural crest cells that originally migrated to more than one branchial arch (Köntges & Lumsden 1996). While it has commonly been thought that patterning information established in the neural tube is brought into the branchial arches by migrating neural crest cells, this finding has been interpreted as evidence that patterning of these skeletal elements is imposed on neural crest cells after their emigration from the neural tube (Köntges & Lumsden 1996; Hunt *et al.* 1998). The fact that toothplates in fishes are not always in register with visceral arch skeletal elements (e.g. the upper pharyngeal toothplates of garfish and bowfins; Nelson 1969) suggests that modules of the dentition might also contain neural crest cells from more than one branchial arch.

Couly & Le Douarin (1990) mapped regions of the ectoderm ('ectomeres') of early chick embryos that maintain their register with migrating cranial neural crest cells as both move ventrad. While the extent to which these units contain patterning information relevant to the dentition or other structures is unknown, ectoderm in the chick likely to be homologous to that which gives rise to upper incisors in mammals is not contiguous at early somite stages with ectoderm homologous to that from which the remainder of the mammalian dentition is probably derived. While different rostrocaudal levels of zebrafish endoderm have been mapped to different regions of the blastoderm stage embryo (Warga & Nüsslein-Volhard 1999), the data collected were on a coarser scale than individual branchial arches and patterning along this axis of the endoderm is poorly understood in vertebrates as a group (Grapin-Botton & Melton 2000).

Much of the recent work on patterning of the branchial arches has focused on *Hox* codes, the specific combination of genes of the *Hox* family of transcription factors expressed by each (reviewed by Hunt & Krumlauf 1992; Hall 1999; Le Douarin & Kalcheim 1999). It has been suggested that there is a tight causal link between this *Hox* code and the final morphology of the branchial arches (Hunt *et al.* 1998). However, identifying the time at which neural crest cells become determined is complicated by the fact that *Hox* genes are expressed in neural crest from before the onset of migration, in branchial arch ectoderm and, to a lesser extent (in terms of number of genes), in pharyngeal endoderm (Hunt & Krumlauf 1992; Hall 1999; Le Douarin & Kalcheim 1999; Grapin-Botton & Melton 2000). While there have been conflicting data on the degree of plasticity of cranial neural crest with respect to *Hox* gene expression, Trainor & Krumlauf (2000) suggested that the *Hox* code and rostrocaudal identity of neural crest cells is initially specified prior to migration and is maintained by permissive signals from the cranial mesoderm. Whether the *Hox* code is involved in specifying modules within the dentition is unknown. The mandibular arch and medial nasal processes, the only structures giving rise to teeth in mammals, do not express *Hox* genes. While *Hox* genes are expressed by the hyoid and posterior gill arches of fishes (Prince *et al.* 1998*a*,*b*; Ahn & Gibson 1999), whether they are expressed in association with pharyngeal teeth is unknown.

Much of the debate over whether neural crest is patterned prior to migration has focused on the morphology of branchial cartilages. As pointed out by Smith & Coates (2000), it is possible that cartilage, bones and teeth are patterned separately, so that even if cartilage identity is determined by the rostrocaudal origin of neural crest cells, this need not be the case for teeth. Indeed, tissue recombination experiments in mammals suggest that the patterning information for tooth initiation and tooth type (i.e. module) is present in the oral ectoderm prior to epithelial thickening (Lumsden 1988; Kollar & Mina 1991). As discussed below, the earliest molecular signals associated with these patterning events are also found in the oral ectoderm. A complication to these studies, however, is that patterning of the dentition has long been known to involve a series of reciprocal signals between epithelial and mesenchymal components (Thesleff & Hurmerinta 1981) and determining which tissue provides the earliest signal is difficult. As an indication of this, the ectoderm employed by Lumsden (1988) had already been in contact with neural crest (Le Douarin & Kalcheim 1999) and possibly also pharyngeal endoderm (Imai *et al.* 1998). In amphibians, pharyngeal endoderm appears to be required for the development of teeth, even those derived from ectoderm (see Hall (1999) and included references).

3. DISSOCIABILITY AND THE EVOLUTION OF TOOTH LOCATION

(a) *Patterns in the evolution of tooth location*

A common trend in the evolution of vertebrates is reduction of the dentition, a process that has occurred in numerous lineages independently (Peyer 1968; Huysseune & Sire 1998). While a number of lineages have lost teeth altogether, including turtles and birds, it is more often the case that teeth are lost in some locations and retained in others (figure 2*a*). This latter pattern of loss might be facilitated if the lost teeth were part of a separate module from those retained. However, region-specific tooth loss by itself does not provide evidence for modularity beyond the level of a genetic pathway for making a tooth that can be deployed in different regions.

In teleost fishes, there is a tendency for reduction of teeth in the central part of the oropharyngeal cavity and a concentration of the remaining teeth in oral and pharyngeal 'jaws' at the anterior and posterior ends of the cavity (Gosline 1985; Huysseune & Sire 1998). The zebrafish and other members of the order Cypriniformes represent an extreme example of this, as teeth have been lost from the entire oropharyngeal cavity except for the fifth ceratobranchials. The single marginal row of teeth

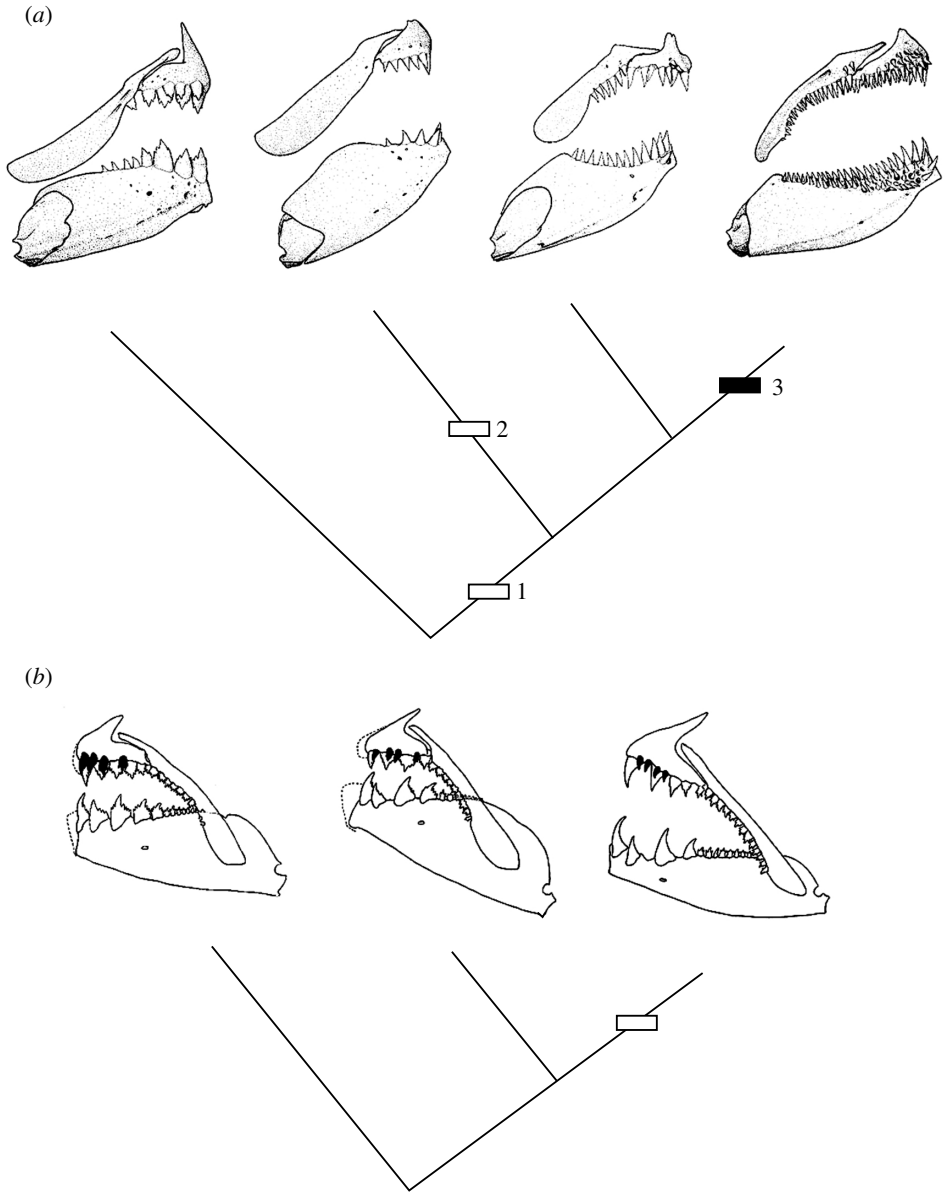

Figure 2. Evolutionary trends in the dentition of characiform fishes. (*a*) Modified from Weitzman & Fink (1985). Oral dentition and phylogeny of four species of the Xenurobryconini (Teleostei, Characidae). From left to right, *Argopleura chocoensis*, *Iotabrycon praecox*, *Xenurobrycon pteropus* and *Tyttocharax madeirae*. Evolutionary losses are represented by open rectangles and gain by a filled rectangle. Event 1, loss of cusps from maxillary, premaxillary and dentary teeth; Event 2, loss of maxillary and posterior dentary teeth; Event 3, spread of teeth beyond occlusal surfaces of maxillae and dentaries. (*b*) Modified from Rosen (1972). Oral dentition and phylogeny of members of the genus *Bramocharax* (Teleostei, Characidae). From left to right, *Bramocharax baileyi*, *B. bransfordi dorioni*, and *B. b. bransfordi*. The open rectangle indicates loss of cusps from anterior teeth of premaxillae and dentaries. Outer row premaxillary teeth are black. Drawings of dentition courtesy of the American Museum of Natural History.

in each jaw of extant mammals is another example of reduction of the dentition, as some mammal-like reptiles possessed teeth on the bones of the palate as well (Kemp 1982; Carroll 1988). In addition, reduction of the number of teeth in the marginal rows is a common phenomenon within the extant orders of mammals (Peyer 1968; Luckett 1993). The laboratory mouse for example, lacks canines and premolars in both jaws, the remaining incisors and molars being separated from each other by a toothless diastema (Ruch *et al*. 1997).

An interesting question, given the widespread reduction of the dentition, is whether lost teeth can be regained. This would represent a violation of Dollo's law of the irreversibility of evolution (Marshall *et al*. 1994) and might be less likely to occur if all of the teeth in a module are lost. It is common to use Dollo's law as an assumption when polarizing tooth location characters for phylogenetic analyses (e.g. Behnke's (1992) analysis of the phylogeny of salmonid fishes). Such an assumption is likely to bias against the identification of instances of the reacquisition of teeth. Nevertheless, a number of potential examples exist in teleost fishes, including the reacquisition of teeth on the third basibranchial in some centrarchids and the parasphenoid of some nandids (Gosline 1985). Johnson & Patterson (1996) suggested that maxillary and gill raker teeth were lost and reacquired in the lineage

leading to the Southern Hemisphere grayling *Prototroctes*, although they pointed out that they knew of no other instances of the reacquisition of such teeth. Reacquisition of teeth has also been proposed to occur in the form of atavisms within species. Examples include the occasional appearance of a second molar in the lynx (Kurtén 1963) and the appearance of vomerine teeth in a single individual of the sciaenid teleost *Pogonias cromis* (Cione & Torno 1987).

In the case of centrarchid third basibranchial teeth, it has been suggested that 'reacquisition' occurred by the displacement of paired and more dorsally located teeth on the hypobranchials (Gosline 1985). If so, one wonders whether hypobranchial and basibranchial teeth form part of the same module. If teeth can only be reacquired in cases where other teeth in the same module still exist, then this might provide an explanation for why oral teeth have never been regained in the cypriniform fishes, despite the existence of piscivorous lineages among the over 2500 species (Nelson 1994). Perhaps the pharyngeal tooth module cannot be redeployed in the oral region. If so, then loss of an entire module of the dentition might represent a developmental constraint on evolution of the dentition.

The extensive dentition of primitive vertebrates leaves little room for truly novel deployment of teeth among extant groups. One potential example is provided by the 'oesophageal' teeth of stromateoid fishes (Haedrich 1967). Others are the teleost lineages with extraoral teeth described above. None of these groups (catfishes, herrings and billfishes) is closely related to each other or is considered to represent basal lineages of teleost fishes. In addition, all of these families with extraoral teeth are related to families without such teeth. While the odontodes of primitive bony fishes such as coelacanths and *Polypterus* might possibly represent the retention of an ancestral feature of vertebrates, similar interpretation of teleost extraoral teeth would require an enormous number of evolutionary loss events in other lineages. More likely alternatives are that they represent the re-expression of the developmental genetic programme for making odontodes (requiring the persistence of this feature despite the absence of its phenotypic expression), or the ectopic expression of the programme for making oral teeth. Comparisons of the development and fine structure of oral and extraoral teeth have been carried out by Huysseune & Sire (1997) for armoured catfishes and Sire *et al.* (1998) for *Denticeps*. In the former case, both types showed similar fine structures with slight differences in development and mode of attachment. In the case of *Denticeps*, teeth and odontodes were extremely similar in size, shape, structure and development. Sire *et al.* (1998) concluded that the odontodes of *Denticeps* represent the expression of teeth in extraoral locations. A possible example of an intermediate stage in such a process in an unrelated lineage is provided by the characiform teleost genus *Tyttocharax* (figure 2*a*). In this taxon, teeth project outside of the mouth because of the formation of additional rows of teeth on the external surfaces of the premaxillary and dentary bones (Weitzman & Fink 1985). Sire *et al.* (1998) interpreted the extraoral teeth of billfishes and armoured catfishes as the result of a similar process to that which they proposed for *Denticeps*. Such a process was proposed to have occurred earlier in the former two lineages because of greater morphological divergence in the extraoral teeth.

Evolutionary tooth loss may occur by a number of general mechanisms. One such mechanism is the failure of tooth replacement, the cause of the absence of teeth in adult sturgeons (Bemis *et al.* 1997). In cases where the primary dentition does not appear, tooth germs may arrest at a variety of stages. In birds, molecular and morphological evidences suggest that tooth development progresses to the stage of epithelial thickening and is then arrested (Helms *et al.* 1997; Huysseune & Sire 1998; Chen *et al.* 2000). The diastema of rodents presents a case where loss of specific teeth has occurred by more than one mechanism. In the lower jaw of the laboratory mouse and a vole, there is no trace of tooth initiation in the diastema, but in the upper jaw, diastemal tooth rudiments progress to the bud stage before arresting in development (Keränen *et al.* 1999). In the zebrafish oral region, no morphological sign of tooth development has been reported (Miyake & Hall 1994; Huysseune *et al.* 1998). The likely differences in mechanism of tooth loss indicated by these examples suggest that in considering possible genes involved in evolutionary changes in tooth location, it is necessary to treat both tooth initiation mechanisms as well as molecular interactions regulating later stages of tooth development. Similarly, addressing mechanisms of tooth gain requires considering whether altering the distribution of tooth initiation signals is sufficient or whether factors required for later stages of tooth development must be altered in their distribution as well. The former situation would be more consistent with a view of modules as autonomous pathways of development that can be activated by simple genetic switches (Raff 1996).

(b) *Models for the genetic control of tooth initiation and early epithelial–mesenchymal interactions*

Neubüser *et al.* (1997) proposed that the earliest step in tooth initiation is the induction by the epithelially localized signalling factor FGF8 of the transcription factor *Pax9* in the underlying mesenchyme. These authors demonstrated that the induction of *Pax9* by FGF8 was antagonized by the signalling factors BMP2 and BMP4. According to their model, FGF8 was expressed in a broader domain than that which gave rise to teeth, with localization of teeth further refined by epithelially expressed BMP4 and mesenchymally expressed BMP2 (figure 3*b*). As pointed out by Jernvall & Thesleff (2000*a*), *Pax9* induction cannot be the only step in tooth initiation because teeth in *Pax9*-deficient mice progress beyond initiation to the bud stage (Peters *et al.* 1998). A recently described tissue-specific knockout of the *Fgf8* gene provides evidence for modularity in tooth initiation (Trumpp *et al.* 1999). Inactivation of this gene in mandibular epithelium resulted in the absence of molars and their associated *Pax9* expression domain, but rudimentary incisors (and their *Pax9* expression domain) were present. These authors suggested that another member of the FGF family might be responsible for tooth initiation in the incisor region. Two other *Fgf*s, *Fgf9* and *Fgf10*, have been shown to be expressed in oral epithelium prior to tooth initiation, and at least the

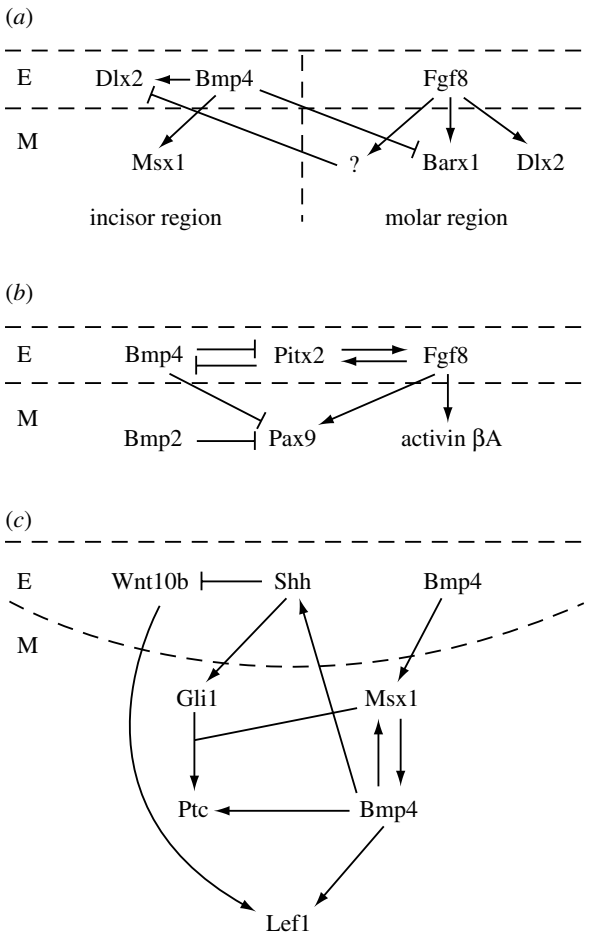

Figure 3. Gene interactions in successive stages of tooth development in the mouse. (*a*) Tooth type determination. Based on Tucker *et al*. (1998*b*) and Thomas *et al*. (2000). (*b*) Tooth initiation. Based on Peters & Balling (1999) and St Amand *et al*. (2000). (*c*) Signalling between thickened dental epithelium and underlying mesenchyme. Based on Peters & Balling (1999), Dassule & McMahon (1998) and Zhang *et al*. (1999, 2000). E, epithelium; M, mesenchyme.

former is capable of inducing some of the same molecules in the underlying mesenchyme as FGF8 (Tucker *et al*. 1999; Kettunen & Thesleff 1998; Kettunen *et al*. 2000). A detailed description of the relationship among *Fgf8*, *Fgf9* and *Fgf10* expression patterns is not available; such data might indicate whether *Fgf8* expression overlaps with that of one or both of the other two genes in the presumptive molar but not incisor regions.

Given the above model of tooth initiation, the regulation of *Fgf8* and *Bmp4* expression is of interest with respect to the control of tooth location. In the chicken, *Fgf8* is expressed in ventral ectoderm prior to the arrival of the neural crest cells that will populate the mandibular arch, and this gene has been proposed to specify the location of the structure (Shigetani *et al*. 2000). Although the mechanism of induction of *Fgf8* expression at this early stage remains unknown, it presumably does not involve signals from the neural crest cells. At slightly later stages (but prior to the morphological appearance of the mandibular arch), *Fgf8* expression appears to be negatively regulated by BMP4 (Shigetani *et al*. 2000). According to these authors, *Fgf8* and *Bmp4* expression remain in relatively fixed positions through the appearance of the mandibular arch, where they are involved in the later event of dentition patterning. Nevertheless, it is possible that regulation of expression of these genes during dentition patterning employs different mechanisms than those involved in initiation of expression. One possibility for regulation of later expression is through signals from underlying neural crest cells, as proposed by Francis-West *et al*. (1998). Transcription factors that may be components of such signalling are *Prx1* and *Prx2* (Lu *et al*. 1999*a*; Ten Berge *et al*. 1998). A dual knockout of these transcription factors, which are localized in distal mandibular mesenchyme, results in the ectopic expression of *Fgf8* (Ten Berge *et al*. 1998). Another potential regulator of *Fgf8* expression is the transcription factor *Pitx2*, whose epithelial expression pattern prefigures the location of teeth (Keränen *et al*. 1999) and which has been shown to be required for tooth development to progress beyond the epithelial thickening or bud stage (Lu *et al*. 1999*b*; Lin *et al*. 1999). Interestingly, *Pitx2* regulates *Fgf8* expression in a positive feedback loop and that of *Bmp4* in a negative feedback loop (Fig. 3B; Lu *et al*. 1999*b*; Lin *et al*. 1999; St Amand *et al*. 2000).

A number of genes are expressed in the earliest dental epithelial thickening in mice, including the transcription factors *Dlx2* (Thomas *et al*. 2000; Zhao *et al*. 2000), *Msx2* (MacKenzie *et al*. 1992) and *Pitx2* (Mucchielli *et al*. 1997) and the signalling molecules *Bmp4* (Vainio *et al*. 1993; Neubüser *et al*. 1997; Tucker *et al*. 1998*a*), *Shh* (Hardcastle *et al*. 1998; Dassule & McMahon 1998) and *Wnt10b* (Dassule & McMahon 1998). However, the signals responsible for epithelial thickening remain unclear (Tucker & Sharpe 1999). *Pitx2* (Lu *et al*. 1999*b*; Lin *et al*. 1999) and *Dlx1*/*Dlx2* (Qiu *et al*. 1997; Thomas *et al*. 1997) knockout mice experience an arrest of tooth development subsequent to epithelial thickening. Early lethality in knockout mice has hampered the analysis of the roles of *Bmp4* and *Shh* in early tooth development (Chiang *et al*. 1996; Åberg *et al*. 1997), and the conditional inactivation of *Shh* in the oral epithelium that has been reported occurred at a stage subsequent to the initiation of epithelial thickening (Dassule *et al*. 2000). It is possible that epithelial thickening is under the control of early *Fgf8* and *Bmp4* expression, as *Pitx2* is positively regulated by FGF8 and negatively regulated by BMP4 (St Amand *et al*. 2000), while *Dlx2* is positively regulated by BMP4 (Thomas *et al*. 2000). If so, then functional redundancy may be contributing to the absence of knockout mice that do not initiate epithelial thickening.

Members of the HH, BMP and WNT signalling families localized in the thickened dental epithelium have been shown to induce the expression of a large number of transcription factors in the underlying mesenchyme, including some (*Dlx1*, *Dlx2*, *Gli2*, *Gli3*, *Pax9*, *Msx1*) whose inactivation in mice causes an arrest of tooth germs at the epithelial thickening or bud stages of tooth development (Bei & Maas 1998; Peters & Balling 1999; Tucker & Sharpe 1999; Jernvall & Thesleff 2000*a*; Fig. 3C). Interestingly, BMP4, which appears to inhibit tooth development at early stages, is an inducer of molecules required for tooth development at the epithelial thickening stage (Vainio *et al*. 1993; Tucker *et al*. 1998*a*). Where examined, most of the molecules involved in interactions between

the thickened epithelium and underlying mesenchyme appear to be expressed in both molar and incisor tooth germs. This includes molecules such as *Msx1* (MacKenzie *et al*. 1992; Tucker *et al*. 1998*a*) and *Dlx* genes (Qiu *et al*. 1997; Thomas *et al*. 1997; Zhao *et al*. 2000) that are regionally restricted prior to the appearance of tooth germs.

(c) *Mutations with restricted pleiotropic effects within the dentition of mice and humans*

Most knockout mice with effects on the dentition cause an arrest of tooth development at either the epithelial thickening or bud stage (Jernvall & Thesleff 2000*a*). In the case of an *Msx1*/*Msx2* dual knockout (Bei & Maas 1998) and knockouts of *Lef1* (Van Genderen *et al*. 1994) and *Pax9* (Peters *et al*. 1998), all teeth are affected equally. However, knockouts with effects limited to specific types of teeth also have been reported. In addition to the inactivation of *Fgf8* described above, these include a *Dlx1*/*Dlx2* dual knockout, in which upper molars fail to progress beyond the epithelial thickening stage while all other teeth are normal (Thomas *et al*. 1997), a knockout of *Activin* β*A*, in which upper molars are normal, but all other teeth fail to progress beyond the bud stage (Ferguson *et al*. 1998), a dual knockout of *Prx1* and *Prx2*, in which mandibular incisors are arrested at the bud stage (Lu *et al*. 1999*a*; Ten Berge *et al*. 1998), a dual knockout of the transcription factors *Gli2* and *Gli3*, in which all teeth are affected, but mandibular incisors most severely so (Hardcastle *et al*. 1998), and a knockout of *Pitx2*, in which development of upper teeth arrests later (bud stage) than lower teeth (epithelial thickening stage, Lu *et al*. 1999*b*).

Restriction of pleiotropic effects of mutations within the dentition is also observed in a number of human syndromes caused by heterozygosity for loss of function alleles at characterized genes. In the case of two independent mutations in *MSX1* (Vastardis *et al*. 1996; Van den Boogaard *et al*. 2000), affected individuals most frequently lacked upper and lower permanent second premolars and upper and lower third molars. Permanent canines and all deciduous teeth were always present, while other teeth frequently missing were lower (but not upper) first molars and upper (but not lower) first permanent premolars. A similar but not identical pattern of tooth agenesis characterized a family with a mutation in *PAX9* (Stockton *et al*. 2000). All molars were frequently missing and the next most common types of missing teeth were second permanent premolars (upper and lower) and first lower permanent incisors. Canines, second permanent incisors and all deciduous teeth were always present. A somewhat reciprocal pattern was seen in the case of mutations in the transcription factor *TBX3*, which have been reported to be associated with agenesis of canines, but not other teeth (see Bamshad *et al*. (1997) and included references). A mutation in *PITX2* (*RIEG*) has been shown to be involved in Rieger's syndrome (Semina *et al*. 1996), which may include dental anomalies, such as the missing upper second incisors and small lower first incisors of an individual reported by these authors. In the above examples, boundaries between affected and unaffected teeth separate teeth in different classes, teeth within classes, upper and lower teeth at the same position, and teeth of different generations (although it is worth noting that permanent molars are thought to represent the same generation as deciduous teeth of the other classes; Luckett 1993). Not included in these examples are agenesis of both members of a tooth family.

Restriction of pleiotropic effects of mutations is one of the hallmarks of modularity (Wagner 1996; Wagner & Altenberg 1996). However, the nature of modularity in the dentition revealed by the above examples in humans can be interpreted in at least two different ways, as pointed out by Thesleff (1996). One is that genes such as *MSX1* form part of a code specifying the identity of modules. Alternatively, as the teeth affected in the *MSX1* example are the last to form, it is possible that reduction of levels of functional MSX1 protein has cumulative affects on tooth development that eventually cause the later-forming teeth to fall below a developmental threshold. This latter view is more consistent with the effects on the entire dentition seen in mice homozygous for mutations in *Msx1*, *Pax9* and *Pitx2*. The numerous pleiotropic effects of mutations in these genes on structures outside of the dentition suggest that amino-acid substitutions inactivating the proteins they encode are an unlikely means of effecting the loss of specific types of teeth. Instead, downregulation of their expression, either in the dentition in general or in specific teeth (if corresponding *cis*-regulatory elements exist), might cause dissociable evolution of different teeth.

(d) *Experimental induction of ectopic teeth*

The novel deployment of teeth or their reacquisition would be facilitated if these events required only the ectopic activation of an initiation signal. There exists a small amount of genetic data from the mouse bearing on this possibility. Ectopic expression of the transcription factor *Lef1* in the lip furrow of a single transgenic mouse resulted in the formation of an ectopic tooth (Zhou *et al*. 1995). *Lef1* is normally expressed in the epithelium of the developing tooth, where it is required for progression beyond the bud stage (Kratochwil *et al*. 1996). Although *Lef1* expression in dental mesenchyme appears to be downstream of members of the BMP and WNT families of signalling molecules, epithelial *Lef1* was not induced by these molecules at the stages examined (Dassule & McMahon 1998). Thus it is unclear if ligands from either of these families might similarly be capable of inducing ectopic teeth. Application of SHH protein in mandible culture has been shown to induce ectopic epithelial invaginations (Hardcastle *et al*. 1998), but whether these represent tooth germs is unclear due to the length of the organ culture experiments. In contrast to the misexpression of the former two molecules, mutations inactivating mouse *Pax6* lead to additional upper incisors in the normal tooth row (Kaufman *et al*. 1995; Quinn *et al*. 1997). Stock *et al*. (1997) and Weiss *et al*. (1998*a*,*b*) speculated that this might represent altered epithelial patterning, but this would have to occur well before tooth initiation.

(e) *Potential genetic mechanisms for the evolution of tooth location*

Tissue recombination experiments in amphibians (Ten Cate 1995) and birds (Kollar & Fisher 1980; Kollar & Mina 1991) suggest that the entire dentition can be lost by a failure of epithelial–mesenchymal interactions. While

frog tadpoles do not develop true teeth consisting of dentine and enamel, urodele larvae do. Transplantation of frog neural crest *in vivo* into urodele larvae resulted in the formation of chimeric tooth germs, while transplantation of urodele neural crest into tadpoles failed to do so (Wagner 1955). Henzen (1957) found that oral epithelium from urodele larvae was capable of inducing true teeth in the frog. As pointed out by Ten Cate (1995), these experiments have been interpreted in opposite ways by different authors, i.e. that frog epithelium has lost the competence to respond to neural-crest-derived signals versus frog epithelium has lost signals responsible for the induction of teeth. Although both hypotheses indicate that tooth loss in frogs has occurred by modification of the epithelium, distinguishing between them requires resolution of where the earliest signals for tooth initiation are located, a problematic issue, as described in § 2d. Modification of oral epithelium as a mechanism of tooth loss is also suggested by the ability of mouse oral epithelium to induce the formation of chimeric teeth when combined with chicken mandibular mesenchyme (Kollar & Mina 1991). That tooth location in vertebrates is more likely to be determined by epithelial competence than that of neural crest is suggested by the ability of oral epithelium and/or endoderm to induce teeth in trunk neural crest in mice (Lumsden 1988) and axolotls (Graveson *et al.* 1997). In the latter case, the ectodermal and endodermal tissues were taken from a stage well before their contact with neural crest.

Some clues as to the genes involved in these instances of evolutionary tooth loss are beginning to appear. In the case of the chicken, *Shh* expression marks what probably corresponds to a dental lamina (Helms *et al.* 1997) and earlier markers of dental epithelium (*Fgf8*, *Pitx2*) and mesenchyme (*Pax9*, *Barx1*) exhibit expression similarities with orthologous genes in the mouse (Francis-West *et al.* 1998; Barlow *et al.* 1999; Chen *et al.* 2000). Chen *et al.* (2000) noted that epithelial *Bmp4*, as well as mesenchymal *Msx1* and *Msx2*, exhibited restricted expression in the chicken mandible compared with that of the mouse. Earlier experiments had indicated that beads soaked in BMP2, BMP4, and BMP7 were capable of inducing the ectopic expression of *Msx1* in chicken mandibular mesenchyme (Wang *et al.* 1998, 1999), and Chen *et al.* (2000) were able to obtain epithelial downgrowths in chicken mandibles treated with BMP4 that resembled those of cap-stage mammalian tooth germs. The latter group of authors concluded that loss of epithelial *Bmp4* expression was likely to be one of multiple evolutionary events leading to tooth loss in birds. In contrast to this example of loss of the entire dentition, Keränen *et al.* (1999) investigated the loss of portions of the dentition by comparing the expression of 11 genes (functioning in BMP, FGF, HH and WNT pathways) in the rudimentary diastemal teeth of the upper jaw of mice and voles with that in the corresponding molars. These authors found that the earliest major difference between the two types of teeth was the downregulation of *Pax9* in the diastemal mesenchyme. They suggested that this was a downstream effect of subtle alterations in the expression of *Fgf8* and *Bmp4* in the oral epithelium.

That there are likely to be multiple pathways to evolutionary tooth loss is suggested by the two examples above, as well as by the presence of signalling molecules of the BMP, HH and WNT families in the early oral epithelium (Dassule & McMahon 1998). Region-specific tooth loss may be due to the reduction of the expression domains of any of these molecules or the loss of oropharyngeal expression of a particular member of one of the families that induces a subset of the dentition. While no example of this latter phenomenon is known, the presence of incisors in mice lacking mandibular *Fgf8* expression (Trumpp *et al.* 1999) suggests this as a possibility. The number of genes that must have their expression domain expanded to result in tooth gain or reacquisition remains unknown. Dassule & McMahon (1998) suggest that teeth are the only location in the oral region where the expression of all three families of signalling molecules overlaps. Whether the expression of all of them must independently be expanded to form teeth in new location is unknown. *Lef1*, whose ectopic expression results in ectopic teeth, is regulated by both BMP and WNT signalling, but the signals regulating its expression in epithelium are unknown.

4. DISSOCIABILITY AND THE EVOLUTION OF TOOTH MORPHOLOGY

(a) *Differences in tooth morphology within the dentition*

Teeth within a dentition may differ from each other in numerous ways. Smith & Coates (2000) list a number of examples in primitive fishes in which teeth differ from adjacent oropharyngeal denticles in histology, including degree of mineralization of dentine, and presence or absence of enameloid caps. In rodents, only the outer surface of the incisors is covered with enamel, in contrast to molars in which the entire crown is so covered (Peyer 1968). Teeth within a dentition may also differ in their mode of attachment to underlying bones. Fink (1981), in a survey of tooth attachment mode in fishes, pointed out that attachment mode may differ within a dentition, but teeth of single bones always share the same attachment mode. Teeth with different attachment modes may occur on adjacent bones, however, as is the case for splenial and dentary teeth of the lower jaw of *Polypterus*. In mammals, some teeth may grow throughout the life of the individual while other teeth in the same dentition do not. Examples include the ever-growing incisors of rodents, incisors (tusks) of elephants, and canines (tusks) of pigs (Peyer 1968). Tooth replacement patterns may also differ within a dentition. In most mammals, two generations of teeth occur at incisor, canine and premolar positions, while molars are not replaced (Peyer 1968). Virtually nothing is known of the genetic control of the above features of teeth, so they will not be considered further. However, it is possible that at least some of the differences described are regulated by genes discussed below in the context of control of tooth identity.

Tooth size and shape differences within a dentition may be discontinuous or graded. An example of discontinuous shape differences between widely separated teeth is provided by the characiform fishes, in which teeth of the oral jaw margins are frequently multicuspid, while teeth of the gill arches are unicuspid (Roberts 1969). More closely spaced teeth also frequently exhibit discontinuous

shape differences. An example is the mammalian dentition, with its frequently chisel-shaped incisors, conical canines and multicuspid premolars and molars. These categories are referred to as tooth classes and the presence of shape discontinuities as heterodonty (Peyer 1968). Within these tooth classes, tooth size and shape form gradients such that neighbouring teeth are more similar to each other than to more distantly located ones (Butler 1995). These gradients apply to the degree of development of particular cusps, roots, crown outline and size, and are not necessarily aligned with respect to the particular teeth in which they reach their maximum degree of development (Osborn 1978; Butler 1939, 1967). Size gradients are also widespread in homodont dentitions, i.e. those consisting exclusively of similarly shaped (frequently unicuspid) teeth (Peyer 1968). An exception to graded differences in shape within mammalian tooth classes is the existence of complex shearing carnassials in carnivores (Peyer 1968). These teeth are the last premolar in the upper jaw and the first molar in the lower jaw and form a shape discontinuity in the postcanine teeth. The derived nature of these teeth suggests the evolutionary independence of members of the same tooth class and might be viewed as the appearance of a new module in evolution.

(b) *Evolution of tooth size and shape differences within the dentition*

In the course of evolution, gradients of tooth size and shape may change their slope as well as their position relative to individual teeth (Butler 1939, 1995). Such changes include homeosis, in which a tooth in a particular location of one dentition comes to resemble a tooth in a different location in a related dentition. Homeosis is often associated with a shift in location of the dentition relative to the rest of the skull (Butler 1941, 1995) and provides evidence for the independence of tooth initiation from the specification of tooth identity or shape. Homeosis in mammals more frequently involves members of the same tooth class but it may occur across classes as well, as in the case of molariform premolars of horses and incisiform canines of cows (Butler 1978*b*).

While shape changes in the dentition may affect all teeth, it is frequently the case that only a subset of the teeth are affected, a phenomenon consistent with the existence of modules in the dentition. Examples of correlated and independent evolution of the dentition in characiform fishes are shown in figure 2. In a clade consisting of *Iotabrycon*, *Xenurobrycon* and *Tyttocharax*, all of the ancestrally multicuspid teeth in the jaw marginal dentition have become unicuspid (Weitzman & Fink 1985; figure 2*a*). Furthermore, a reversal of this condition has occurred in *Xenurobrycon heterodon*, which affects only the anterior teeth of the upper and lower jaws. Interestingly, this is roughly the same set of teeth that has lost its cusps in an unrelated clade of characiform fish of the genus *Bramocharax* (Rosen 1972; figure 2*b*). While the evolution of the teeth of the upper and lower jaws appears to be correlated in these examples, teeth at corresponding positions of the upper and lower jaw may also evolve independently. In the unrelated characiform genus *Alestes*, the ancestral condition of multicuspid teeth with all cusps in a line has been retained in the lower jaw, but modified in the upper jaw into teeth with a more circular arrangement of cusps (Roberts 1967). An example of independent evolution of upper and lower teeth in mammals is provided by the appearance of an incisiform canine and a caniniform premolar in the lower but not upper jaw of the mole (*Talpa*, Luckett 1993). Correlated and independent evolution of upper and lower teeth has also occurred in the pharyngeal region of characiform fishes. The ancestral condition for this group is the presence of unicuspid pharyngeal teeth, which have acquired multiple cusps in the chilodontid genera *Caeotropus* and *Chilodus* (Vari 1983). The former genus has upper and lower pharyngeal teeth with the same morphology, while in the latter, more cusps are found on upper pharyngeal teeth than lower ones.

In addition to the evolutionary changes discussed above, teeth exhibit homeosis and correlated change in their variation within species (Bateson 1892, 1894; Kurtén 1953). In general, they appear to obey 'Pearson's Law' (Levinton 1988), in which adjacent teeth are more strongly intercorrelated with respect to size than more distant teeth (Kurtén 1953). Interestingly, Kurtén (1953) found that in some cases, the correlation between occluding teeth was stronger than between adjacent teeth. In addition, teeth with a reduced size (e.g. vestigial teeth), relative to those in other species, tended to lose their correlation with other teeth.

(c) *Models for the genetic control of tooth identity and shape*

Two main classes of models have been proposed to explain the existence of mammalian tooth classes with gradients of tooth shape and size within them. A 'gradient field' model (Butler 1939, 1995) proposed that tooth size and shape were controlled by morphogen gradients in the jaws that were external to developing tooth germs. The existence of at least three tooth classes was explained by the proposal that there were at least three separate morphogen gradients. An alternative 'clone' model (Osborn 1978) explained the different tooth classes as being derived from separate clones of mesenchymal cells and the shape gradients as the result of changes in the growth properties of the mesenchyme as teeth were initiated sequentially. Kollar & Mina (1991) proposed that the initial information for determining tooth type resides in the oral epithelium based on their observation that epithelium from the incisor region of early mouse mandibles can instruct molar region mesenchyme to form incisiform teeth when cultured intraocularly. The elucidation of the role of *antennapedia*-class *Hox* genes in the specification of the identity of insect segments led to proposals that similar codes of homeobox genes might be involved in specifying the identity of mammalian tooth classes (Weiss 1990, 1993; Sharpe 1995). The 'odontogenic homeobox code' of Sharpe (1995) postulated that members of several non-*Hox* homeobox gene families were involved in specifying tooth identity based on their regionalized expression in mesenchyme along the axis of the dentition.

Recent support for the control of tooth type by epithelial signalling was provided by Tucker *et al.* (1998*b*; figure 3*a*). These authors found that expression of *Bmp4* in distal mandibular arch epithelium and *Fgf8* in proximal mandibular arch epithelium corresponds to presumptive incisor and molar regions, respectively. Presumptive

incisor mesenchyme was marked by *Msx1* expression and molar mesenchyme by the expression of the transcription factor *Barx1*. Tucker *et al.* (1998*b*) demonstrated that FGF8 was capable of inducing the expression of *Barx1* and that BMP4 inhibited this expression. Treatment of mandibular explants with beads soaked in noggin protein (an inhibitor of BMP signalling) resulted in loss of *Msx1* expression (previously known to be induced by BMP4, Vainio *et al.* 1993; Chen *et al.* 1996; Tucker *et al.* 1998*a*) and the expansion of *Barx1* expression into the presumptive incisor region. Most importantly, noggin-soaked beads were shown to cause the formation of teeth with molariform shape from explanted regions normally giving rise to incisors. The authors proposed that tooth type was determined by signalling molecules with regionalized expression in the mandibular epithelium through their induction of regionalized expression of transcription factors in the underlying mesenchyme. As they pointed out, it remains unclear whether shifts in the expression of *Msx1* or *Barx1* are sufficient for the transformation of tooth type. It is noteworthy that the same molecules proposed to determine tooth type by Tucker *et al.* (1998*b*) have also been proposed to specify tooth location (Neubüser *et al.* 1997). If both these and other hypotheses on the function of *Bmp4* are correct, this gene would have the opposing activities of specifying incisor identity (figure 3*a*; Tucker *et al.* 1998*b*), inhibiting tooth initiation (figure 3*b*; Neubüser *et al.* 1997) and inducing odontogenic mesenchyme (figure 3*c*; Vainio *et al.* 1993; Tucker *et al.* 1998*a*). The apparent paradox may be resolved by hypothesizing that these different activities occur sequentially in the order listed. In support of this, Tucker *et al.* (1998*b*) point out that induction of *Pax9* expression occurs later than that of *Barx1*.

While the reduced dentition of the mouse provides little opportunity for studying the genetic control of gradients of tooth shape (a single incisor and molar are present in each jaw quadrant at the stages most often studied), the results of Tucker *et al.* (1998*b*) do provide potential insight into this issue. Specifically, in some of their experiments employing noggin-soaked beads, they obtained teeth intermediate in shape between molars and incisors. It is tempting to speculate that such teeth develop from a lesser degree of inhibition of BMP signalling than those more fully transformed into a molariform shape and therefore that gradients in the dentition reflect a graded distribution of BMP proteins.

The crown shape of teeth is believed to be determined by the folding of the interface between the epithelium and mesenchyme (Butler 1956). Significant progress has been made in identifying molecular signals controlling folding of tooth epithelium (MacKenzie *et al.* 1992; Jernvall 1995, 2000; Jernvall *et al.* 1998; Keränen *et al.* 1998; Jernvall & Thesleff 2000*b*, Jernvall & Jung 2000). Clusters of non-dividing epithelial cells known as 'enamel knots' have been found to be the source of a number of signalling molecules, including members of the FGF and BMP families as well as the transcription factor *Msx2*. Enamel knots may regulate the folding of the epithelium by ceasing to divide themselves while stimulating neighbouring cells to divide. In multicuspid teeth such as molars, the first forming (primary) enamel knot is not associated with a cusp, while all subsequently forming (secondary) enamel knots are. Differences in cusp number between incisors and molars are reflected in the absence of secondary enamel knots in the former while differences in cusp pattern between mouse and vole have been shown to be associated with differences in the number and location of enamel knots (Jernvall 1995; Keränen *et al.* 1998). Regulation of tooth shape would therefore be a matter of regulating the timing and location of appearance of enamel knots (Jernvall 1995; Jernvall & Thesleff 2000*b*; Jernvall & Jung 2000). How such regulation is accomplished to provide shape differences within a dentition is unknown; it has been speculated that this might occur by some sort of reaction-diffusion mechanism (Weiss *et al.* 1998*a*; Jernvall & Thesleff 2000*a*; Jernvall & Jung 2000). The primary enamel knot has been proposed to be induced by mesenchymal signals, potentially including BMP4 (Jernvall *et al.* 1998), but the link between this event and the earlier determination of tooth type remains a mystery (Jernvall & Thesleff 2000*a*).

(d) *Potential genetic mechanisms for the evolution of tooth shape*

As mentioned above, the phenomenon of homeosis in variation and evolution implies that determination of tooth identity and tooth location are uncoupled. It is therefore somewhat surprising that both processes appear to make use of the same epithelial signalling molecules, i.e. BMP4 and FGF8. If this proves to be correct, then homeosis is likely to be the result of shifts in the location of expression of these molecules at specific temporal stages. Butler (1995) has described the phenomenon of homeosis as the shift of tooth location relative to the rest of the skull. Two species differing in this manner might exhibit identical distributions of epithelial signalling molecules at early stages when tooth identity is determined while the later expression of these molecules (responsible for determining tooth location) differs. Significantly, *Fgf8* and *Bmp4* have also been proposed to play a role in the outgrowth and patterning of facial primordial (Francis-West *et al.* 1998). Butler's (1995) observation that teeth and bones often evolve in concert might reflect the response of both structures to the same patterning information, e.g. the regional expression of transcription factors in the mesenchyme. If tooth and bone identity were determined at the same time by the same mechanism, this would provide a basis for Butler's (1995) observation that teeth occupying the same relative position in the skull of two species frequently exhibit the same morphology regardless of their numerical position in the tooth row.

Shifts in the position of transcription factors determining tooth identity relative to the locations of tooth initiation, as proposed above, parallel the proposal that the homeotic evolution of crustacean appendages has occurred by the alteration of the spatial expression pattern of *Hox* genes (Averof & Patel 1997; Gellon & McGinnis 1998). An additional parallel between arthropod segments and the mammalian dentition is that both have evolved according to Williston's law (Williston 1914; Gould 1977), which states that meristic series evolve by the reduction in number of elements and the appearance of a greater differentiation in the morphology of the

Table 1. Evolutionary and genetic evidence for modularity in the vertebrate dentition.

potentially separate modules	morphological discontinuity/ evolutionary independence	restricted pleiotropy/genetic independence[a,b]
dentition versus dermal denticles	stingray dentition versus stinging spines (Compagno 1999) *Polypterus* odontodes versus teeth (Meinke 1982)	no relevant data
individual teeth within a class	carnivore carnassials versus other molars and premolars (Peyer 1968)	*MSX1*: 1st and 3rd versus 2nd molars (Vastardis *et al*. 1996) *PAX9*: 1st versus 2nd incisors (Stockton *et al*. 2000) *PITX2*: 2nd versus 1st incisors (Semina *et al*. 1996)
tooth generations	primitive perissodactyl premolars versus milk molars (Butler 1952) characiform larval unicuspid versus adult multicuspid teeth (Roberts 1967)	*MSX1*: permanent versus deciduous premolars, permanent versus deciduous incisors (Vastardis *et al*. 1996; Van den Boogaard *et al*. 2000) *PAX9*: permanent versus deciduous premolars, permanent versus deciduous 1st incisor (Stockton *et al*. 2000)
tooth families	perissodactyl molarized milk molar and premolar versus unmolarized milk molar and premolar (Butler 1952)	no evidence
teeth attached to single bones	mammal upper incisors versus other teeth (Luckett 1993) placodont premaxillary versus maxillary teeth (Carroll 1968) *Landonia* premaxillary versus maxillary teeth (Roberts 1973) *Polypterus* splenial versus dentary teeth (Fink 1981)	*Activin* βA: upper incisors versus upper molars (Ferguson *et al*. 1998) *Dlx1*/*Dlx2*: upper molars versus upper incisors (Thomas *et al*. 1997)
subsets of teeth attached to the same bone	mammal tooth classes of dentary (Luckett 1993) placodont tooth classes of dentary (Carroll 1968) *Landonia* multicuspid versus blade-like dentary teeth (Roberts 1973) *Bramocharax bransfordi bransfordi* unicuspid anterior dentary and premaxillary teeth versus multicuspid posterior dentary and premaxillary teeth (Rosen 1972)	Bmp inhibition: lower incisors versus molars (Tucker *et al*. 1998*b*) *Fgf8*: lower molars versus incisors (Trumpp *et al*. 1999) *Prx1*/*Prx2*: lower incisors versus molars (Lu *et al*. 1999*a*; Ten Berge *et al*. 1998) *MSX1*: molars, premolars, incisors versus canines (Vastardis *et al*. 1996; van den Boogaard *et al*. 2000) *PAX9*: molars, premolars, incisors versus canines (Stockton *et al*. 2000) *TBX3*: canines versus molars, premolars, incisors (Bamshad *et al*. 1997)
upper versus lower teeth at the same position	*Talpa* lower versus upper canine, premolar (Luckett 1993) *Alestes* premaxillary versus dentary teeth (Roberts 1967) *Chilodus* upper versus lower pharyngeal teeth (Vari 1983)	*Activin* βA: lower versus upper molars (Ferguson *et al*. 1998) *Dlx1*/*Dlx2*: upper versus lower molars (Thomas *et al*. 1997) *Gli2*/*Gli3*: lower incisors more severely affected than upper (Hardcastle *et al*. 1998) *Pitx2*: lower teeth more severely affected than upper (Lu *et al*. 1999*b*) *Prx1*/*Prx2*: lower versus upper incisors (Lu *et al*. 1999*a*; Ten Berge *et al*. 1998) *MSX1*: lower versus upper 1st molar, upper versus lower 1st premolar (Vastardis *et al*. 1996), upper versus lower 2nd incisor, lower versus upper 1st incisor (Vastardis *et al*. 1996; van den Boogaard *et al*. 2000) *PAX9*: lower versus upper 1st incisor (Stockton *et al*. 2000) *PITX2*–upper versus lower 2nd incisor, lower versus upper 1st incisor (Semina *et al*. 1996)
ectodermal versus endodermal teeth	characiform oral versus pharyngeal teeth (Roberts 1969)	no relevant data
teeth arising from single branchial arches	characiform oral versus pharyngeal teeth (Roberts 1969)	no relevant data

[a] Teeth listed as affected versus unaffected.
[b] Human genes indicated by all upper-case letters, mouse genes by upper-case letter followed by lower case.

remaining segments. This differentiation would represent individualization of repeated elements, and hence the evolution of modularity, by a process known as 'parcellation' (Wagner 1996). It has been proposed that differentiation in the segments of insects has occurred through changes in the interaction of *Hox* genes with their downstream targets, rather than the appearance of new *Hox* genes or changes in their expression pattern (Warren *et al*. 1994; Carroll *et al*. 1995; Gellon & McGinnis 1998). An analogous process may have been responsible for the evolution of mammalian heterodonty. This event occurred in the mammalian lineage after its divergence from other amniote lineages (Kemp 1982). The chicken, whose toothed ancestors did not have a heterodont dentition (Carroll 1988), exhibits similar restriction of *Fgf8*, *Bmp4*, *Msx1* and *Barx1* along the proximal distal axis of the mandible as seen in mammals (Mina *et al*. 1995; Francis-West *et al*. 1998; Barlow *et al*. 1999; Shigetani *et al*. 2000; Chen *et al*. 2000). Therefore differences in tooth shape within the mammalian dentition may have made use of patterning information already present in the jaw, presumably also used by skeletal elements. This may explain why it has been possible for heterodonty to evolve in parallel in other vertebrate lineages, such as crocodilians, characiform fishes and others (Roberts 1969, 1973; Carroll 1988; Kieser *et al*. 1993; Huysseune & Sire 1998).

5. CONCLUSIONS

It is likely that the vertebrate dentition is organized into modular groups of teeth based on the criteria of discontinuous shapes within a single dentition and independent shape change in evolution. In addition, the dentition itself is almost certainly a module of the dermal exoskeleton. Table 1 summarizes this evidence along with that for the independent genetic control of these phenotypically defined modules. Although both types of evidence generally overlap in the types of module identified, much additional data are required to demonstrate specific cases of links between genetic and evolutionary modules. Nevertheless, it is still possible to speculate on the genetic basis of modularity and dissociation in the development and evolution of the dentition. That teeth in different regions of the dentition can be lost independently may be explained by differences in tooth initiation signals in the molar and incisor regions of the mouse (Trumpp *et al*. 1999). Another potential explanation is regional variation in the strength of inductive and inhibitory signals, as proposed from studies of rudimentary teeth in the diastema of mice and voles (Keränen *et al*. 1999). The observation that genes involved in epithelial–mesenchymal interactions subsequent to tooth initiation can have tooth-type-specific effects when inactivated provides another potential mechanism for region-specific tooth loss, especially in cases in which tooth loss is the result of an arrest in development subsequent to initiation. That altered expression of a single gene is sufficient to induce ectopic teeth (Zhou *et al*. 1995) may explain how it is possible for lost teeth to be reacquired or teeth to be deployed in novel regions in the course of evolution. If this is the case, then entire modules need not be recreated or built up from scratch.

Studies of the genetic basis of tooth-type selection (Tucker *et al*. 1998*b*) suggest that at least some modules in the dentition might be specified by the action of selector genes in the mesenchyme, which are themselves induced by signalling molecules in the odontogenic epithelium. Although no evidence is available from the dentition, it is possible that modification of the control by these selector genes of downstream 'realisator' genes would allow modules of the dentition to evolve as a unit and independently from other modules. Homeotic changes in the evolution of the dentition might be explained by the shift of selector gene expression domains relative to locations at which teeth are initiated. That tooth initiation and determination of tooth identity may involve the same molecules expressed at different times provides opportunities for both independent and correlated evolution of tooth identity and location. Homeotic changes between tooth classes suggest that individual teeth (identified by location and timing of initiation, Luckett 1993) might become parts of different modules in the course of evolution.

In addition to modularity in the dentition that might result from homeobox codes, restricted pleiotropy and the capacity for evolutionary independence of different teeth might be the result of a patterning cascade mechanism, as proposed by Jernvall (2000) and Jernvall & Jung (2000), for the control of molar cusp patterns in mammals. In such a mechanism, slight alterations in parameters, such as levels of gene expression, can have large effects on late-developing structures (cusps or teeth) with only slight effects on ones that develop earlier. Significantly, such genes might be required in the development of all teeth, while effects of mutations might be seen only in those that develop later, as is the case in the human genetic dental defects described above. A patterning cascade mechanism might therefore cause late-developing teeth to be largely independent in evolution from those that develop earlier. In addition it might provide an explanation for size and shape gradients within mammalian tooth classes (the members of which appear sequentially in development, Luckett 1993) as well as provide a means of gradient alteration in evolution.

In cases where modules do correspond to differences in gene expression between sets of teeth, it would be useful to know whether they tend to be controlled by different members of the same gene family, by members of different gene families, or by separate *cis*-regulatory elements within single genes (the latter case would then push the question one step higher in a regulatory hierarchy). The model for tooth type determination of Tucker *et al*. (1998*b*) suggests that members of different gene families are involved but does not rule out, for example, additional paralogues of *Barx1* whose roles are as yet undiscovered.

6. APPROACHES TO THE FURTHER STUDY OF MODULARITY AND DISSOCIATION IN THE DENTITION

Much of the developmental genetic evidence for modularity in the dentition discussed above has not been collected for the purpose of understanding this phenomenon. Nevertheless, the approaches employed should continue to provide valuable information. These include identification of genes differentially expressed between

incisor and molar regions in mice, construction of knockout mice, application of proteins to cultured odontogenic regions, transgenic misexpression of genes in the oral region, cloning of genes involved in human genetic dental anomalies, and comparison of gene expression between mammalian species with different dental patterns. Studies specifically addressing modularity in the dentition will need to employ these as well as additional approaches, both in terms of organisms and experimental methods.

To date virtually no genetic data are available on the development of odontodes located outside of the oropharyngeal region. As demonstrated by Miyake *et al.* (1999), elasmobranchs (sharks, skates and rays) provide a promising system for comparing gene expression between dermal denticles, true teeth and oropharyngeal denticles. Another group of organisms with potential in this respect are fishes of the genus *Polypterus*, which possess odontodes on dermal bones, scales, fin spines and throughout the oropharyngeal region (Meinke 1982; Jollie 1984). While difficult to breed, some progress has been reported in obtaining embryonic series from spawnings in the laboratory (Bartsch *et al.* 1997).

Teleost fishes provide a valuable opportunity to study the degree to which teeth in different regions of the oropharyngeal cavity constitute separate modules. The zebrafish is an obvious choice for study due to its popularity as a developmental genetic model system (Dooley & Zon 2000). As it possesses teeth in the pharyngeal but not the oral region (Huysseune *et al.* 1998), comparison of gene expression and function in the oral region of this species with that in related species possessing oral teeth allows the study of region-specific loss of the dentition (Stock & Weiss 1998). The lack of oral teeth is, to some extent, a liability, however, in that it is generally not possible to compare the effects of induced mutations on the dentition of different regions. Another teleost model system that may help overcome this limitation is the medaka, *Oryzias latipes*. This species possesses oral and pharyngeal teeth (Parenti 1987; Miyake & Hall 1994) and an extensive repertoire of experimental techniques has been worked out for the analysis of its development (Ishikawa 2000). Particularly promising are mutagenic screens (Ishikawa 2000), which may reveal genes with effects on subsets of the dentition, and large-scale *in situ* hybridization screens (Henrich & Wittbrot 2000), which may reveal genes expressed during the development of certain types of teeth but not others.

Transgenic reporter analysis of *cis*-regulatory regions of genes involved in tooth development represents an experimental approach that should prove useful in the analysis of modularity. For example, Thomas *et al.* (2000) demonstrated that epithelial and mesenchymal expression of *Dlx2* in the developing jaws are regulated by separate *cis*-acting elements. Whether separate elements of other dentally expressed genes control expression in different types of teeth remains to be demonstrated. Analyses of *cis*-regulatory elements from one species in a related model organism (see, for example, Shashikant *et al.* (1998), for the evolution of the vertebral column) might reveal whether differences in expression relevant to dental pattern are due to differences in *cis*- or *trans*-acting factors.

An additional methodology that might be applied profitably to the study of dissociable evolution of the dentition is quantitative trait locus (QTL) analysis (Lynch & Walsh 1998). Teleost fishes represent a promising system for this approach because of the large numbers of offspring that can be obtained from single crosses and the fact that numerous closely related species differ in dental pattern. For example, a number of species within the teleost family Centrarchidae can produce F_2 generations (Childers 1967; Whitt *et al.* 1971) and differ from each other in tooth location, as well as tooth shape (Birdsong & Yerger 1967; Gosline 1985). While it is difficult to go from QTLs to cloned genes (Darvasi 1998) detailed linkage maps being constructed for several teleost species (zebrafish, Kelley *et al.* 2000; medaka, Naruse *et al.* 2000; rainbow trout, Young *et al.* 1998; Sakamoto *et al.* 2000; tilapia, Kocher *et al.* 1998) should facilitate this process.

As data on the genetic basis of modularity and dissociation in the development and evolution of the dentition continue to accumulate, it will be of interest to compare them with those gathered from other meristic systems such as the segment of arthropods. Given that rules such as 'Williston's law' have been applied to such systems, one might ask whether there are similar generalizations that can be made at the developmental genetic level. At this point, significant progress would have been made toward confirming William Bateson's 100-year-old insight that meristic systems provide a valuable window into the mechanisms of evolution.

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