Animal Domestication and Vertebrate Speciation: A Paradigm for the Origin Of Species

by

Susan Janet Crockford
B. Sc., University of British Columbia, 1976

A Dissertation in Partial Fulfillment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Interdisciplinary Studies

Supervisor: Dr. Quentin X. Mackie

Abstract

Heterochrony (changes in developmental rates and/or timing) has been successfully argued as the most significant process in evolution. Heterochronic differences between species are recognized in many vertebrate lineages, including hominids and domesticates. However, the biological mechanism(s) that initiate and control heterochrony have not yet been identified. Here I address the issue via an interdisciplinary perspective and extensive literature review. Although domestic animals have traditionally been defined as products of human innovation, this view has fallen out of favour as untenable; I offer a testable hypothesis to explain the initial domestication of most animals as natural heterochronic speciation, a concept I then apply to all vertebrates, including hominids. Integral to this hypothesis is evidence that thyroid hormones (THs, T_3 / T_4) are essential regulators of all traits that undergo change during domestication, strongly implicating THs as pivotal to this process. While a hormonal basis for heterochronic speciation has long been suspected, a major role for THs has not been suggested before now: here I present the first comprehensive theory for the role of THs in vertebrate evolution.

THs are known to be secreted in a distinctly pulsatile manner, so that blood concentrations of THs, when measured frequently over the course of a day, display profiles with distinct peaks and valleys. Daily *TH rhythms* (THRs) appear to be species-specific, with species-distinct ontogenic shifts; individual variation in THRs also appear to exist. Due to the critical known effects of THs on ontogenic development, and known interactions with other hormones, I propose that individual variations in genetically-controlled THRs (*THR phenotypes*) must generate the coordinated individual variation in morphology, reproduction and behaviour within populations known to be essential for evolutionary change. I suggest that during many speciation events, a non-random subset of individuals splits off from an ancestral population (a subset who share manifestations of *one particular* THR phenotype), drastically reducing the total variation of THR phenotypes in the founder population; this loss of THR diversity results in rapid and well-coordinated changes in descendants. This paradigm provides a robust and testable theoretical framework for determining how animals transform over time in response to environmental change.

Table of Contents

List of Tables

List of Figures

Acknowledgements

For some people, an endeavour like this really does begin in childhood; it certainly did for me. I must begin by acknowledging the contributions made by my mother, Barbara - who taught me that boxes were meant to be opened - and my father, Ken, who bought me the Alaskan Malamute I desperately craved as a child. Although I'm sure he didn't understand why it was so important to me to have this particular type of dog, he bought it anyway - and it really did change my life. That wolf-like dog, and the other Malamutes who replaced her over the years, became in-your-face and at-your-feet reminders of the growing list of questions I had about domestication, speciation and evolution. Eventually, I had to do something about that list and this document is the result. My father didn't live long enough to have any inkling of what he'd set in motion, but my mother was there to cheer me on much of the way, although sadly not to the end.

I want to thank wholeheartedly all those persons who engaged in debate (sober or not) on the many topics covered in this dissertation, including friends, colleagues, close relatives and a few total strangers: I could not have produced this manuscript without you (especially Gay Frederick, Cairn Crockford, Rebecca Wigen, Margot Wilson, the late David Moyer, Ranald Donaldson, Dan Kareem, Josephine Joorgens-Dogger and Toby Carter). For passing along essential leads early in my quest to pull the concept together, I thank: Darcy Morey, Ray Coppinger, Nicolas Rolland, and George Mackie. From the last few years of updating and expansion, I thank in particular: Jerome Dobson, the late David Horrobin, Alec Hercbergs, Iain McKecknie, and Josephine Joorgens-Dogger. Lastly, for pure enthusiastic support, encouragement, and guidance when needed, I extend warmest thanks to Quentin Mackie, Nicolas Rolland, Nancy Turner, Barry Glickman, Valerius

Geist, the late Etian Tchernov, Kenneth McNamara, Nancy Minugh-Purvis, Atholl Anderson, the late Peter Hockachka and the late David Horrobin. I am indebted to Michael McKinney (University of Tennessee, Knoxville) for active and enthusiastic support at several critical stages.

Last but not least, I want to acknowledge the encouragement of my children, Jesse and Laura McMillan, who bore the brunt of my drive to bring this concept to fruition. The journey dominated our lives for more than ten years and I thank them for their unfailing and unconditional support; I know I wouldn't have been able to finish without it.

Chapter 1 Background and Current Paradigms

"Any scientific revolution or synthesis has to accept all sorts of black boxes, for if one had to wait until all black boxes are opened, one would never have any conceptual advances."

Ernst Mayr (1991:146) on Darwin's inability to suggest how species originate

1.1 Introduction

Charles Darwin (1859) published the first widely-read description of the world as an ever-changing place and provided the foundation for the modern science of evolution as the study of organisms transforming over time and space. Darwin demonstrated that species did indeed change and even suggested why, but he couldn't say *how*. What is astounding, however, is that almost 150 years later the question remains incompletely answered; the "black box" in Darwin's argument has become evolution's enigma. Modern evolutionary biologists have not, to date, been able to suggest an explicit biological mechanism that fully accounts for how species and higher level taxa originated.

Species are considered the essential units of evolution, the critical end products of natural selection (Otte and Endler 1989; Mayr 1982, 1988). Understanding how species transform from one entity into another, in all lineages, is as important to studies of the past as it is to the present. Eldridge and Gould's (1972) concept of *punctuated equilibrium,* for example, was presented more than 25 years ago to explain patterns of speciation evident in the fossil record (*punctuated equilibrium* refers to recurring bouts of very rapid speciation interspersed with periods of relative stasis in organismal form). Despite compelling indications that punctuated equilibrium is a real and significant phenomenon (Gould and Eldredge 1977, 1993), we have not yet been offered a precise

biological explanation of how such an evolutionary pattern could be attained, even given 30 years of dedicated attention by Gould himself (2002).

Heterochrony (changes in the rates of developmental growth and/or shifts in the timing of developmental events) has been successfully argued as the most probable process by which the rapid speciation changes inherent to the concept of punctuated equilibrium could occur. As a consequence, heterochrony may be the most significant process in evolution (Richardson 1995). Heterochrony may affect the initiation and cessation of growth stages and/or implement changes in the rate of foetal and/or postnatal growth of the ancestral species to various degrees, making possible a wide variety of shape and size differences in descendant populations. In vertebrates, heterochrony has been implicated in a number of evolutionary novelties indicative of macroevolutionary change (that is, characteristics different enough to warrant placing the taxon into a distinct genera or family, as defined by Mayr 1982), such as bipedal morphology in hominids (Berge 20002) and the marine adaptations of cetaceans (Berta and Sumich 1999; Thewissen 1998). Heterochrony is also implicated in many instances of microevolutionary change (that is, characteristics that distinguish species or adaptations that occur within populations, as defined by Mayr 1982) (Geist 1986, 1998; McKinney 1988; McKinney and McNamara 1991). However, the precise biological mechanism (or mechanisms) responsible for the initiation and/or implementation of heterochronic changes have not been determined (McKinney 1998; McNamara 1995, 1997; Voss and Shaffer 1997) and this leaves serious gaps in our understanding of evolution (Mayr 1994, 1996; 2001).

I suggest that we have been unable to determine the process that initiates rapid speciation changes because we lack an appropriate paradigm with a suitable, testable

hypothesis. The population genetic models that served well for decades in explaining other aspects of evolutionary change (as reviewed by Rice and Hostert 1993; updated by Gavrilets 2003) are simply not adequate for addressing heterochrony. The interactions between genotypes and phenotypes that affect developmental processes are not linear, one to one relationships (as population genetic models assume) but complex webs of interdependence (Alberch 1985a, 1991; Finch and Rose 1995; Hall 1992, 2003; Maynard Smith 1983; Maynard Smith et al. 1985; West-Eberhart 2003). While many biologists have been cognizant of this fact, including a pioneer of population genetics, Sewell Wright (Provine 1986), they have so far been unable to supply a paradigm that successfully takes these complex relationships into account (e.g. Dufty et al. 2002; Goodnight and Wade 2000; Lovejoy et al. 1999; Streelman and Danley 2003; Wu et a. 2003).

I present a novel approach to investigating the role that heterochrony plays in evolution by taking a critical and in-depth look at the process we call *domestication*. Prompted by evidence that domestic mammals include some of our best known examples of heterochronic change, I argue that domesticates do not always result from one continuous process initiated by humans. Instead, I suggest the process is very often comprised of two distinct parts, the first of which (*protodomestication*) is initiated by the animals themselves. Furthermore, animals that have undergone protodomestication share certain morphological, physiological and behavioural similarities indicating that thyroid hormones (THs) played a pivotal role in the heterochronic changes that occurred. Understanding how THs exert their centralized control over essential biological functions and other hormone-producing organs provides the foundation for a model that can explain

protodomestication as a natural speciation process. The model I present to explain the role of THs in protodomestication also explains, in remarkable detail, precisely how all vertebrate species and higher level taxa could transform over time in response to changing environmental conditions.

This paradigm is based on the testable hypothesis that particular physiological phenotypes - variations in TH metabolism that occur naturally among individuals comprising a species - are the critical characteristics targeted by natural selection. Due to the profound influences that THs exert on embryonic and postnatal growth in particular, many distinctive morphological and behavioural traits characteristic of new species may simply be inevitable consequences of selection for individuals with certain physiological phenotypes of TH metabolism. Selection for any one trait controlled by a particular TH metabolism variant effectively selects for all behavioural, physiological and morphological manifestations of that TH variant. As THs are intimately involved in the biological mechanism that allows individuals to adapt their body functions, in a coordinated fashion, to environmental conditions that vary over the short term (daily or seasonally), I propose that the same mechanism allows populations of individuals (*species*) to adapt to environmental conditions that change over evolutionary time scales.

A new perspective such as this is long overdue in evolutionary biology. The theory provides a plausible biological mechanism to account for broad-scale evolutionary transformations in response to environmental change and is exceptionally powerful because it is based on an explicit biological hypothesis that can be tested experimentally. As a consequence, the concept provides an unusually strong theoretical framework for future research. It constitutes a significant *paradigm shift*, a term defined by Kuhn (1970)

and further elucidated by Root-Bernstein (1989), because it looks at the question of how species evolve from a completely different perspective than the population genetic models that have dominated the field until now (reviewed in Mayr 1982; Schwartz 1999). By augmenting rather than challenging the basic tenets of accepted evolutionary theory, this paradigm more accurately reflects and predicts the complex nature of inter- and intra-specific relationships we are now able to discern using phylogenetic analysis methods.

The end result of reassessing domestication as a natural speciation process is an elegant paradigm for heterochronic speciation that explains in precise detail the truly dynamic relationship that exists between animals and their environment, and thus, between individual variation and evolutionary transformations in response to environmental change over time. The concept describes, for the first time, a plausible biological mechanism to account for the gradual adaptation of species to local environmental conditions, and for the rapid transformation of one vertebrate species to another in response to environmental change or colonization of a new habitat (i.e. microevolution). In addition, the mechanism explains how some evolutionary novelties characteristic of new genera and families (i.e. macroevolution) may contribute some unique "blips" to the patterns of heterochronic change that dominate the fossil record.

1.2 Speciation and Evolution

An obvious place to start in a discussion of how species originate as a result of heterochronic processes is a definition of *species*. What should be a simple and straightforward exercise, however, taps into one of the most hotly debated and long-standing controversies in biology (e.g. Holliday 2003; Mallet 1995; Otte and Endler

1989) – a controversy I suggest is largely a consequence of our inability to explain how species come to be in the first place, and which will not likely disappear until the underlying issue is resolved.

Many people, including biologists, have been taught that there is one definition for the concept of *species* that works for all organisms, when nothing could be further from the truth. The most widely cited definition is a slightly simplified version of Mayr's (1982) *biological species concept*: "a species is a group of organisms that can actually or potentially interbreed with one another, but not with other species." Mayr first advanced this definition in 1942, as a contribution to the so-called "modern evolutionary synthesis" (a group project that was orchestrated to merge the new discipline of genetics with Darwinian principles). Despite wide-spread acceptance and use of Mayr's definition over the years, few people really stop and think about the fact that it cannot be applied to extinct (fossil) taxa or to species that reproduce asexually (as many plants do). Many people also get the impression that any evidence of natural hybridization between two populations automatically invalidates their distinct species status, which is simply not true (Mayr has repeatedly insisted that occasional hybridization does not invalidate his definition (e.g. 1982: 274, 284; 1991: 148) (see also Arnold 1997, and Section 3.2). More importantly, perhaps, is the unstated implication that all such exceptions are of little consequence, which is also not true.

In fact, a number of distinct definitions for the concept of *species* have been advanced over the years, each of which has some short-comings. The most widely known are definitions based strictly on one group of features or another, such as morphology (*typological species* and *evolutionary species* – good for palaeotologists and taxonomists,

often problematic for field biologists); reproductive life history characteristics (*biological species*- good for vertebrate field biologists, almost useless for botanists or palaeontologists); genetic traits (*phylogenetic species* – good for field biologists, only occasionally – and recently - useful for palaeontologists) (reviewed in Mayr 1988, 1991). Less well known definitions combine several aspects of species traits, such as Templeton's (1989) *cohesion species concept* (combining morphology and life-history) or Mallet's (1995) *genotypic cluster definition* (combining morphology and genetics).

In practice, taxonomists tend to use the definition (or combination of definitions) that best applies to the set of organisms involved (Mayr 1988) and few complain as long as the criteria used are apparent.

For the purposes of this discussion I have chosen to use Alan Templeton's (1989) *cohesion species concept*, which defines a species as "the most inclusive population of individuals having the potential for phenotypic cohesion through intrinsic cohesion mechanisms". Templeton's definition of a species differs somewhat from Mayr's widely-accepted *biological species concept* because it emphasizes the mechanisms that induce reproductive cohesiveness within discrete populations rather than stressing mechanisms that prevent reproduction between them. It also explicitly includes morphological criteria ("phenotypic cohesion") as well as reproductive life history traits ("intrinsic cohesion mechanisms"), while Mayr's deals with reproductive life history traits only. The differences between the two may appear rather small but they are significant.

Also, Templeton's *cohesion species concept* emerges from his stated conviction that in attempting to understand speciation as an evolutionary genetic process "...speciation, regardless of the precise definition of species, is best approached

mechanistically by examining the evolutionary forces operating on individuals within populations or subpopulations and tracing their effects upward until they ultimately cause all of the members of that population or subpopulation to acquire phenotypic attributes conferring species status on the group" (1989:12). Since this is the approach I use here in examining the relationship of heterochrony to domestication and speciation, I find the cohesion species concept the most applicable.

This definition, as Templeton points out, explicitly ascribes species status to members of *syngameons* (a group of closely-related species occupying similar or adjacent habitats, common in many plant families) as well as to genera composed of closely related plant and animal species who can and do produce hybrids, but who nevertheless each possess distinct morphological, ecological, genetic and evolutionary characteristics - as is the case for many domestic animals, including members of the wolf/dog genus (*Canis*).

In contrast to the vociferous debate involved in defining species, relatively little conflict exists regarding the basic theoretical concepts of how new species arise. Species not only evolve over time (transform); they multiply (become more numerous). New species arise, as Mayr (1988) has summarized, primarily when an ancestral population splits, a process due either to changing physical aspects of the environment (*geographic* or *allopatric* speciation), or via the budding off and modification of a peripherally isolated founder population (*peripatric* speciation). Although probably much less common, two neighbouring populations of a single species may gradually diverge until they become two distinct species (*parapatric* speciation, also called *phyletic gradualism*). The decision of where to draw the line between one species and another in cases of

parapatric speciation is necessarily somewhat arbitrary. Finally, although controversial, division of populations may occasionally occur within the same area via ecological, or host, specialization (*sympatric* speciation).

Mayr (1991:147) has stated quite emphatically that "speciation is never merely a matter of genes or chromosomes but also of the nature and geography of the populations in which the genetic changes occur; geography and the genetic changes of populations affect the speciation process simultaneously." Thus, species change in response to their environment and any proposed biological mechanism for speciation must account for this interaction. However, as Mayr (pers. comm., September 2003) explains further "a whole population, including whole species, is never reflecting a typological entity. What is selected are aggregates of individuals." Thus, a mechanism to explain speciation must also ascribe a role for the individuals that comprise a population or species.

1.3. Domestication and Evolution

There is as little consensus among anthropologists in defining *domestic* animals (O'Connor 1997) as there is among biologists in defining *species*. For example, Reed's (1984) definition is simple: domesticates are animals whose breeding is, or can be, controlled by humans. Clutton-Brock (1992a) defines a domestic animal as one that has been bred in captivity for purposes of economic profit to a community that maintains a mastery over its breeding, organization of territory, and food; she insists also that animals cannot be domesticated unless they are owned (1992b). Isaac (1970:20) lists five criteria that define fully domestic taxa:

1. the animal is valued and there are clear purposes for which it is kept;

2. the animal's breeding is subject to human control;
3. the animal's survival depends, whether voluntarily or not, upon man;
4. the animal's behaviour (i.e. psychology) has changed as a result of domestication;
5. morphological characteristics appear in individuals of the domestic species which occur rarely if at all in the wild.

Because opinions vary widely on the degree of emphasis the morphological, physiological and behavioural differences between domesticates and their ancestors should have in any definition of domestication, lists of what constitute "real" domestic animals also vary considerably (e.g. Corbet and Clutton-Brock 1984). Some authors include reindeer but not zoo or circus animals (Reed 1984), although the later often exist for generations under total human control; some call Asian elephants *semi-domestic* (Isaac 1970), while others list them as true domesticates (Reed 1984); rats, mice and pigeons are excluded by some (Clutton-Brock 1992a; Isaac 1970; Zeuner 1963) but not others (Berry 1984; Hawes 1984; O'Connor 1997; Robinson 1984c; Tchernov and Horwitz 1991).

Nevertheless, the morphological, physiological and behavioural differences between many domestic mammals and their wild ancestors (alluded to by Isaac's definitions) are well-documented (Asdell 1964; Clutton-Brock 1981, 1992a,b; Olsen 1985; Mason 1984a; Price 1984; Reed 1984; Tchernov and Horwitz 1991; Teichert 1993). Such differences are amazingly parallel, even between unrelated lineages and ecologically diverse taxa. Hemmer (1990) summarizes these differences as:

1. overall body size reduction;
2. shortening of the facial bones of the skull (accompanied by changes in dentition

and horn size reduction in those species with horns);

3. lowered age of sexual maturity;
4. increased docility (lessened fearfulness);
5. increased fecundity (principally through larger litters);
6. changes in dominant coat colour alleles (such as *piebald* and *non-agouti* colouring);
7. changes in reproductive timing (frequency and/or seasonality)

As domesticates do not conform to any single, all-encompassing definition, the traits associated with domestication listed above do not apply unambiguously to every taxon. Nevertheless, the biological differences between wild taxa and their natural domestic descendants can be clearly established for dogs, pigs, cattle, sheep, goats and the lesser-known Asian bovids – water buffalo and mithan. These examples are used here for illustrating the range of ecological and physical types of animals that have undergone similar biological change as a result of domestication.

The major distinctive differences between wild ancestors and domestic descendants have been shown by several recent studies to be the result of changes in developmental rates and/or timing: the heterochronic process associated with the retention of juvenile characteristics known as *paedomorphosis* or (less accurately) *neotony* (Goodwin et al. 1997; Morey 1990, 1992, 1994; Wayne 1986c). Retention of juvenile characteristics are implicated in many of the morphological traits common to all domesticates because of the very nature of the differences: smaller overall size, shortened snout, juvenile behaviour. Other traits (such as increased fecundity, docile behaviour, piebaldness, and polyestrousness), as discussed in more detail below, seem to be associated consequences of paedomorphosis rather than paedomorphic traits themselves.

As there is no real consensus on what constitutes a domestic animal, there is understandable confusion regarding the exact taxonomic and evolutionary relationship that domestic animals have to their ancestors (Corbet and Clutton-Brock 1984; Gautier 1993; Uerpmann 1993). The taxonomic and systematic arguments originate largely from the ambiguous definitions of domesticates, which lump animals that are biologically distinct from their ancestors (and thus probably deserve species status) with taxa that are not, simply because they are all defined by cultural criteria (i.e. "under human control"). Morey (1994) suggests that the ambiguous nature of the definitions stem partly from an inability to separate the intense symbiotic relationship which humans now have with domestic animals (and the diversity this has clearly generated) from the process that produced biologically distinct taxa in the first place.

Lack of consensus in defining domestic taxa is significantly problematic for anthropology, as Crabtree (1993) has noted: without a clear definition of a domestic animal, it is difficult, if not impossible, to investigate the role humans played in the process that produced them. Obviously, not all animals currently under human control got there by the same route; therefore, all domesticated animals should not be treated as equivalent evolutionary entities, even though they may now fill equivalent cultural roles.

I suggest that the process traditionally called *domestication* is actually composed of two distinct parts, summarized here but discussed in more detail later (Section 3.2). I suggest the term *protodomestication* be used for the natural speciation process whereby particular subsets of wild ancestors generate descendants with modified biological features, a process often initiated by the animals themselves. *Protodomestication* occurs within human dominated environments and although it does not necessarily involve direct

human interference, it does create animals that are uniquely amenable to subsequent human manipulation.

Classic domestication is the term I suggest be used to describe the processes of conscious and unconscious human selection (working in concert with natural selection) that modify any captive population into regional varieties and polymorphic breeds after prior *protodomestication*. While these definitions contrast sharply with the traditional view of domestication (which collapses the two stages together for all taxa), I argue that the two processes are so distinct they require different terms of reference.

All traditional definitions of domestication either state explicitly or imply that domesticates are derived from animals deliberately removed from the wild (e.g. Clutton-Brock 1981, 1992a,b; Künzi and Sachser 1999; Rindos 1984; Teichert 1993; Zeuner 1963). The assumption that deliberately captured animals form the source population of domesticates is implicit even in some non-traditional explanations of domestication, such as proposed by Morey (1992, 1994) and Tchernov and Horwitz (1991). Thus, the cultural explanation for domestication tries to convince us that the suite of biological changes common to many domesticates could have been deliberately initiated time and again by culturally diverse humans across a wide variety of taxa. However, I reiterate the point made by Morey (1994): the fact that humans have been able to control and manipulate domesticates so thoroughly and with such dramatic success over the last few thousand years does not prove that the process began with the deliberate intent to do so.

There are serious and significant objections to the idea that humans are responsible for deliberately initiating heterochronic changes in all domesticates, which Budiansky (1992), O'Connor (1997), Coppinger and Schneider (1995), Coppinger and

Coppinger (2001), and Morey (1990, 1992, 1994) address in detail. One objection is the high failure rate for the many known cases of deliberate domestication that have occurred since Egyptian times (Janssen and Janssen 1989). Many species of animals have been deliberately tamed; few of these have been known to breed in captivity and fewer still show heritable biological changes over time as a result (Zeuner 1963). Another obstacle is the technology of confinement. How could our ancestors have managed, in the initial stages particularly, to successfully constrain healthy, breeding populations of sexually-mature wild animals (even if tamed as juveniles), when this practice remains a considerable challenge even today? The most compelling of these objections, however, as Coppinger and Schneider so aptly point out, is that paedomorphic traits and their consequences (such as juvenile or docile behaviour, increased fecundity, small size and piebaldness) could not have been selected for by humans out of wild populations - either consciously or unconsciously - since those traits simply did not exist in the wild populations domesticates were derived from. Paedomorphic changes had to occur *before* selection for those traits could be used to shape future generations.

An exhaustive discourse on the subject can be avoided, however, simply by acknowledging that the most significant objection to the cultural explanation of domestication is that it is not testable. Without refutability, the traditional explanation for how domesticates were initially produced can never be a scientific hypothesis, no matter how persuasive the argument. More importantly, any insight gained in examining the process cannot be applied elsewhere. A more useful approach, which I explain in detail in the final chapter, is to examine domestication in biological terms, as an evolutionary process compared and contrasted with speciation.

1.4 Experimental domestication

An elegant example of the intricate biochemical, physiological, and developmental interactions that are pertinent to both speciation and domestication is provided by a series of selection experiments on foxes that began in the 1950's at the Siberian Institute of Cytology and Genetics in Novosibirsk under D. K. Belyaev (Belyaev 1979, 1984) and continues under the direction of his colleague, L.N. Trut (Trut and Osadchuk 1997). The experiments began with a large population of farmed silver foxes (a naturally-occurring black colour morph of the red fox, *Vulpes vulpes*, native to northern Canada and Alaska). These foxes retained all the characteristics of the wild form, with one coordinated annual breeding cycle (females "seasonally monoestrous," males with active sperm in spring only) and an annual moult. They were also generally timid of people.

The researchers assessed silver foxes from several local fur farms and selected, as an experimental population, individuals that demonstrated noticeably less "fearful" behaviour towards people than their cohorts (about 20% of the total). When approached, these animals reacted with limited curiosity rather than aggression or fear, although they still could not be handled. In silver foxes, the timing of oestrous varies among individuals over a period of several weeks (as is true for virtually all mammals, e.g. Bamfield 1974); females of Belyaev's selected population of less fearful animals turned out to be the earliest breeders of the original total population. This early breeding status of less fearful animals suggests that there was an existing polymorphism for timing of oestrus within the original population that was correlated with the selected behaviour.

After several generations of breeding and selecting for non-fearful behaviour, the

oestrous cycle and timing of the annual moult of many females had receded in the season by several months (i.e. shifting from late February to Decemeber). As the experiment continued, oestrous and moult receded further still until several females were experiencing two oestrous cycles annually, one in the spring and another in the fall. After twenty generations, some females were able to produce two litters per year (it took several more generations for males to "catch up" with females in this propensity to be reproductively active during the fall). This diestrous pattern of reproduction (which occurs in most modern domestic dogs) was found to be inherited not as a recessive characteristic as expected, but as an incompletely dominant trait. The totally surprising result was that after twenty generations, a number of novel traits suddenly appeared: a curled tail, drooping (flop) ears, a coat colour pattern of distinctive brown markings referred to as *brown piebald*, and a classic *white piebald* pattern referred to as *star* (*S*). Inexplicably, all of these traits, once they had appeared, inherited in dominant fashion.

Physiologically, the animals from this last generation had smaller adrenal glands associated with lessened secretion of corticosteroid hormones and increased levels of seratonin (see also Belyaev and Trut 1975; Osadchuk 1997; Trut et al. 1972). Females had higher levels of progesterone and oestradiol in early pregnancy accompanied by higher fertility than the original group. The pineal glands of these animals were physically smaller, suggesting to some of the researchers that this might account for the changes in reproductive seasonality (Kolesnikova 1997). Subsequent osteometric analysis of selected foxes revealed that minor changes had occurred in cranial conformation, such that males from the selected population were much closer in size and shape to females than to those from a control sample (Trut et al. 1991).

Belyaev (1979) described the animals with these novel morphological and physiological traits as also having remarkably "dog-like" behaviour: they barked and were quite unafraid of people. He concluded that something in the selection for non-fearful behaviour was not only causing paedomorphic (heterochronic) changes in both morphology and behaviour (see also Trut 1988) but was disrupting the normally constrained timing of sexual reproduction. Subsequent research indicated that the changes were not caused by selection for particular structural genes or by spontaneous mutations, leaving the investigators somewhat baffled by the results (Trut 1999).

Selection experiments were extended by the Siberian research group in 1980 to farmed mink (*Mustela vison*) in the same fashion as the foxes (Trapezov 1997). After only 4 years of similar selection in mink, two novel white-spotted colours appeared that were shown to be semi-dominant in inheritance, as *star* and *mottled* patterns are in selected foxes (in contrast, other rare colours and colour patterns in mink are recessive traits that require intense inbreeding to generate consistent expression).

Several comparable selection experiments were carried out in other countries during this same period. Hemmer (1990), working in Germany during the late 1970's on fallow deer, used a coat colour variant rather than behaviour as a selection factor. Melanistic individuals (black variants of the normal coat colour of brown with white spots) were selected by Hemmer over a few generations, which produced animals that were a novel solid brown without spots and noticeably more docile in their behaviour. Similarly, Keeler (1975) studied several specific colour morphs of farmed foxes (*red, silver, pearl, amber, glacier*) in the United States. The production of these colours had been previously determined by experimental breeding to be governed by mutations in

least 3 genes (4 in the case of amber) whose affects appeared to be additive. Keeler found that successively paler colouring in these foxes was accompanied by successively larger animals with disproportionately smaller adrenals and less fearful behaviour.

Coppinger and Schneider (1995:41) recently assessed Belyaev's experiments in their discussion of heterochronic neotony (i.e. paedomorphosis) and its relationship to dog evolution, and stated: "the transformation from wild fox into dog-like creature that Belyaev made in a few generations simply by selecting for tameness must mirror in some way the original transformation of wolf into dog. Belyaev thought that selection for tameness "destabilized" the genome in such a way as to create evolutionary novelties. As we learn more about gene action and biochemistry we find that he was probably not very far from the truth. The diestrous heat cycle and the piebald coat may be results of a neotonic process, but they are not neotonic characters themselves".

Unfortunately, although Belyaev and his colleagues, as well as Keeler and Hemmer, made valiant attempts at unravelling the physiological basis for domestication changes, they were essentially doomed to fail because they were asking their questions too soon. Genetic and biochemical knowledge essential for interpreting the results of their experiments have only become available, piece by piece, within the last ten years.

1.5. Heterochrony and evolution

A convincing argument has been made that heterochrony is a significant, if not the most significant, process in evolution (Gould and Eldridge 1977, 1993; Krumlauf 1994; McKinney and Gittleman 1995; Raff et al. 1987; Richardson 1995) and has probably been so since the Cambrian (Alberch 1991). Heterochrony is much more common as an

evolutionary process than is generally appreciated, with differences resulting from changes in developmental rates and/or timing recognized in the history of many wild taxa, including (among many others): hominids (Minugh-Purvis and McNamara 2002); whales and porpoises (Thewissen 1998); goats, sheep and deer (Geist 1971, 1986); true seals (Berta and Sumich 1999); several fish species (Dickoff 1993; Klingenberg and Ekau 1996); rodents (Hafner and Hafner 1988) and amphibians (Elinson 1987; Reilly 1994; Voss 1995; Yeh 2002).

Heterochrony may affect the initiation and/or cessation of growth stages as well as implementing changes in foetal and/or postnatal growth rates of the ancestral species to various degrees, so that heterochronic changes make possible a wide variety of shape and size differences in descendant populations. Some controversy exists regarding the precise evolutionary consequences of particular heterochronic changes implemented at various stages of development. While summaries by Klingenberg and Spence (1993) and McKinney (1999) have attempted to resolve some of the ambiguity and confusion in applying certain terminology, some definitions used in the literature remain contentious (e.g. Raff 1996). Nevertheless, heterochrony as a general principle has been utilized successfully to explain certain instances of changes that result in new genera or families (i.e. evolutionary novelties or *macroevolution*) as well as the more commonly occurring species-level changes characteristic of *microevolution* (Gould 2002:1039).

As species are the essential units of evolution, the mechanism (or mechanisms) that initiate and implement the processes of speciation must be identified if we are to understand how evolution actually works. This is particularly true for instances of rapid speciation, given the widespread occurrence and the heterochronic nature of such

changes. Changes in gene frequencies of rare mutant alleles, often proposed to explain both microevolution (e.g. Rice and Hostert 1993; Gavrilets 2003) and macroevolution, are simply inadequate for explaining rapid speciation.

The study of genes at the molecular level (*molecular genetics*) has been proposed as a useful tool for the investigation of relationships within groups that share microevolutionary histories (Avise 1989; Novacek 1992, 1993). In many cases, phylogenetic studies have indeed clarified the taxonomic position of certain taxa that were not resolvable by other methods, including (among others): extinct species of New Zealand moas (Cooper et al. 1992); the extinct ground sloth *Mylodon darwinii* (Hoss et al. 1996); the red wolf *Canis niger* (Lehman et al. 1990; Roy et al. 1995; Wayne and Jenks 1991; Wayne and Gittleman 1995); and the red panda *Ailurus fulgens* (Slattery and O'Brien 1995). In a significant number of lineages, however, the relationships remain as confusing as ever because ecological and morphological histories appear to contradict genetic histories.

Talbot and Shields (1996a, b) for example, undertook an extensive and detailed genetic study and found one particular geographic subspecies of brown bear in Alaska (*Ursus arctos*) to be consistently closer to the polar bear (*Ursus maritimus*) than to any other subspecies of Alaskan brown bear (an example discussed in more detail in Section 3.31). Meyer et al. (1990) found that the genetic relationships suggested by their study of Lake Victoria ciclid fishes (family Ciclidae) did not correspond to the taxonomic pattern suggested by morphological criteria (see also Kaufman et al. 1997). Arnason et al. (1995) found closer genetic similarities among morphologically and ecologically diverse Antarctic seal species (family Phocidae) than among morphologically and ecologically

similar Arctic seals. Arnason and colleagues (Arnason et al. 1993; Arnason and Gullberg 1994) also found the molecular difference between the bottom-feeding grey whale (*Eschrichtius robustus,* a species so morphologically distinct it is placed in a family by itself, Eschrichtiidae) and other rorquals (of the family Balaenopteridae) is less than that found among some species *within* the genus *Balaenoptera.*

The examples given above are only a few of many present in the literature. While species-level contradictions are puzzling enough (e.g. the brown bear/polar bear example), the real challenge presented by these studies is to explain how substantial morphological and behavioural differences (such as the macroevolutionary-type changes demonstrated by the grey whale example) can be associated with such small genetic changes.

Even before much was known of the molecular basis for embryonic development, Hedrick and McDonald (1980:94) predicted that "changes in genetic regulation would be the favoured genetic strategy for a population adapting to a sudden and substantial environmental change." Small mutations within regulatory genes that operate during embryonic development are now acknowledged as the most probable mechanism by which large morphological changes could occur without substantial genetic change (Krumlauf 1994; Raff 1996; Raff et al. 1987; Richardson 1995). Some evolutionary novelties are suggested to have arisen as epigenetic side effects of changing rates in developmental processes simply because such shifts can lead to changes in the position and interaction of formerly separate tissues (Müller 1990). Müller suggested that in such cases, the timing of activation of existing genes may be all that is modified (with no genetic mutations in the regulatory genes themselves involved) and that this change in

activation can lead to morphological transformation.

Homeotic complex (*Hox*) genes in embryos have been identified as sites of developmental regulation. For example, *Hox* genes have been found to direct such early embryonic developmental functions as the diversification of vertebrae along the central body axis and digit formation in the limb of vertebrates (Gerhart and Kirschner 1997; Kenyon 1994; Krumlauf 1994; Lawrence and Morato 1994; Lovejoy et al. 1999; Morgan and Tabin 1994; Tickle 1991). Again, although control over both small and large morphological changes that are heterochronic in nature might be attributed to *Hox*-type genes, we still do not know exactly how such change is either initiated or coordinated.

Fortunately, biologists have not heeded Dawkins' (1976:39) warning that "embryonic development is controlled by an interlocking web of relationships so complex that we had best not contemplate it." However, despite other advances, the precise machinery that initiates heterochronic change has not yet been identified, although a hormonal mechanism has long been suspected (De Pablo 1993; McKinney 1998).

Thyroid hormones (THs), in particular, have been implicated in heterochronic speciation in a number of taxa (Härlid and Arnason 1999; Hayes 1997; Jennings and Hanken 1998; Voss 1995) and the adaptive nature of their correlation to heterochrony in responding to environmental change has been demonstrated (Reilly 1994). THs have also been shown to be essential for early embryonic development in a number of taxa (e.g. Flamant and Samarut 2003). Surprisingly, in spite of these harbingers, the broader evolutionary significance of the association of heterochrony with THs have not been actively pursued until now.

CHAPTER 2 THYROID HORMONES AND EVOLUTIONARY CHANGE

2.1. Thyroid function

Aside from a well recognized role in general metabolism (e.g. Franklyn 2000), thyroid hormones are perhaps best known for their essential influence on metamorphosis and adaptive colouration in amphibians and fish (e.g. Grau et al. 1985; Dickhoff 1993; Voss and Shaffer 1997), and in mammalian hibernation (e.g. Tomasi et al. 1998). However, the total range of influence of THs on ontogenic development (from early embryonic through postnatal stages) and adult physiology in all vertebrates is truly staggering. THs are known to be essential for: early embryonic cell migration, differentiation and maturation; both embryonic and postnatal growth; development of the entire central nervous system, including the eyes and brain; hair growth; adrenal gland function; skin and hair pigment production; development and function of the gonads (Dawson et al. 1994, 1996; Evans 1988; Gunaratnam 1986; Longcope 2000; McNabb and King 1993; Naumenko 1973; Nunez 1985; Schreibman et al. 1993; Thommes and Woods 1993; Wurtman et al. 1968). Through a cascade of direct and permissive (ancillary) effects on regulatory genes and basic cell functions (which may also incorporate the influence of other hormones), THs are able to influence virtually all biological functions (e.g. Flamant and Samarut 2003).

The thyroid gland is ubiquitous among vertebrates. It arises early in the vertebrate evolutionary sequence (i.e. is present in chordates onward), emanating from endoderm of the cephalic portion of the alimentary canal of the embryo (Hadley 2000). The thyroid gland (or glands, where it is has become a paired organ) is composed of numerous follicles that store the protein *thyroglobulin*, which act as a substrate for *tyrosine*

iodination (derived from the essential amino acid *phenylalanine*, itself derived from dietary proteins; iodine is an essential mineral derived from dietary sources). Iodine-bound tyrosine derivatives are stored in the thyroid gland until *thyroid stimulating hormone* (TSH) triggers thyroglobulin to be hydrolyzed and released, with four iodine molecules attached, as *thyroxine* or *tetraiodothyronine* (T_4). Unlike many hormones, such as growth hormone and prolactin (Rand-Weaver et al. 1993), the chemical form and structure of the thyroxine molecule is identical among vertebrates and even across phyla (Eales 1997).

Some thyroxine is deiodinized and released from the thyroid gland with one less iodine molecule attached, as *triiodothyronine* (T_3), although most conversion to T_3 occurs in tissues, including the brain and placenta (Bernal 2002, Smallridge and Ladenson 2001). T_3 degrades more quickly (i.e. has a much shorter half-life) but is more metabolically active than T_4. T_3 appears to be the more physiologically relevant form (more active in metabolism and gene regulation), in part due to its greater affinity (10-15X higher than T_4) for binding to receptors (Huang et al. 2001). *In situ*, tissue-specific conversion of T_4 to T_3 appears to be essential for TH-controlled effects in certain tissues, including the pituitary and the brain (Chan and Kilby 2000). However, it has also been demonstrated that T_4 by itself has critical effects on early growth and development, although the precise mechanism of these actions are not well understood (Brent 2000; Brent et al. 1991; Lavado-Autric et al. 2003).

As a gene regulator, T_3 has been found to bind to both nuclear and mitochondrial thyroid hormone receptors to form a *ligand-receptor complex*. This T_3-receptor complex then binds to a specific DNA sequence located within the promoter region (the *thyroid*

responsive element, or TRE) of a number of genes, triggering the transcription of gene products (enzymes and proteins) within cell nuclei and mitochondria (Chan and Kilby 2000; Koibuchi et al. 1996; Shin and Osborne 2003; Wrutniak et al. 2001). Thus thyroid hormone, in its T_3 state, has been found to influence the transcription of a wide variety of genes in rats, including those involved in the synthesis of lung surfactants, nerve and epidermal growth factors, and a number of critical brain function proteins (Garcia-Segura and McCarthy 2004; Kőhrle 2000; Oppenheimer and Schwartz 1997; Raja et al. 1991).

Direct (non-genomic) effects of T_3 have also been demonstrated; that is, some interactions do not involve the binding of the hormone to a receptor or TRE. In particular, these non-genomic effects have been identified in cell and mitochondrial membranes and control several essential functions. For example, T_3 has been found to stimulate Ca^{2+}-ATPase production in cell membranes (Shin and Osborne 2003), increase oxidative phosphorylation in mitochondria (Wrutniak et al. 2001) and induce the synthesis of Na^+/K^+-ATPase needed to activate the so-called "sodium pump" that produces heat in mitochondria (Hadley 2000; Wrutniak et al. 2001). Of particular importance to this discussion is an essential role for T_3 in *steroidogenesis* (the synthesis of steroid hormones from a cholesterol substrate, which takes place in mitochondrial inner membranes and is required for manufacture of all glucocorticoids, catecolamines, testosterone, and oestrogen). T_3 has been shown to stimulate *steroidogenic acute regulatory protein* (*StAR*) gene expression in a time and dose-dependent manner (increasing production of the enzyme needed to convert cholesterol into pregenolone, the first step in steroid hormone manufacture) in Leydig cells of testes of male rats and thus significantly increasing testosterone production (Manna et al. 1999). Although this has yet to be proven a general

phenomenon affecting all steroidogenic cells, THs have been shown to regulate StAR protein-mediated steroidogenesis in adrenal cortex tissue (Jefcoate 2002) and to induce gonadal growth in Japanese quail (Yoshimura et al. 2003).

Both THs bind to several plasma proteins for circulation in the bloodstream. In humans and mice, these TH-binding proteins are *thyroxine-binding globulin* (TBG), *serum albumin*, and *transthyretin*. Transthyretin is particularly important in moving THs within cerebrospinal fluid (Gagneux et al. 2001) and also transports retinoic acid, a vitamin A derivative (Episkopou et al. 1993). The recognition of transthyretin as a common carrier molecule for both THs and retinoic acid may be significant to several biochemical, physiological and developmental functions common to both. In particular, the molecular structures of THs and retinoic acid are especially similar in the region of their nuclear receptor DNA binding sites (Morita et al. 1990) (see Section 3.34).

THs are secreted into the blood stream by the thyroid glands when *thyrotropin releasing hormone* (TRH) from the hypothalamus induces the release of *thyroid stimulating hormone* (TSH or *thyrotropin*) by the pituitary gland (Harris 1959). TSH released by the pituitary gland initiates TH secretion by the thyroid glands (see Crockford 2003, Appendix B; Figure 1). In non-mammalian vertebrates, *corticotropin-releasing hormone* (CRH, or *corticotropin*), which in mammals appears to act exclusively on adrenal tissue, also elicits release of TSH and subsequently, TH secretion (Denver 1999; De Groef et al. 2003): this secondary control mechanism over TH release has been shown to give amphibians in particular an important additional set of hormonal cues for regulating metamorphic processes that can be exquisitely timed to rapidly changing environmental conditions (such as the sudden drying or flooding of a pond).

THs are released in a rhythmic manner in all vertebrates because the stimulus the pituitary gland receives from the hypothalamus (via TRH) is pulsatile (Greenspan et al. 1986, 1991; Haisenleder et al. 1992; Scanlon and Toft 2000). Thus, in response to pulsatile TRH-regulated TSH stimulation, THs are secreted into the bloodstream in a distinctly rhythmic manner in a wide variety of vertebrate animals, including humans (see Table 2.1, Part I, section 20).

Hormone pulsatility originates high in the hormonal cascade, where electrical stimulation of receptors in the retina and *central nervous system* (CNS) relay signals to the pineal gland (a small organ in the brain) and the hypothalamic region of the brain (which forms the walls and lower part of the third ventricle). The pineal is well known for its ability to translate electrical signals into biochemical messages (e.g. neurohormones, such as *melatonin*, *serotonin*, and *noradrenalin*): pulses of neurohormones produced by the pineal gland in response to electrical stimulation from the retina and CNS prompt intermittent stimulation of the hypothalamus to secrete hormone-releasing hormones, including TRH, that are relayed down the hormonal cascade (Haisenleder et al. 1992; Wright 2002). Although the pineal gland was once thought entirely responsible for sensing changing environmental conditions of all kinds and initiating appropriate physiological responses (Hadley 2000; Korf 1994), it now appears this is not the case.

Recent evidence has been presented that a direct neural connection exists between the *suprachiasmatic nucleus* (SCN) of the hypothalamus and the retina in mammals. The direct SCN-retinal connection provides an alternate control mechanism in these vertebrates (and perhaps others) for secretion of both neurohormone and hormone-releasing hormones, including TRH, that is capable of by-passing or augmenting the

control exerted by the pineal gland via melatonin secretion (Reppert and Weaver 2002; Scheer et al. 2001). Thus, pulsatile release of pineal melatonin *or* direct stimulation of the hypothalamus can stimulate pulsatile secretion of TRH. TRH pulses kindle bursts of TSH secretion from the pituitary, and TSH pulses stimulate pulsatile release of THs from the thyroid. Pulsatility may be essential to physiological activity of all neurohormones, as continued exposure of tissues to melatonin, for example, soon makes them unresponsive to increased levels of hormone (Hadley 2000; Haisenleder et al. 1992).

The precise frequency and amplitude of TH pulses are known to change both seasonally and daily according to other physiological demands in all vertebrates (Cogburn and Freeman 1987; Ferguson 1994; Gancedo et al. 1996; Gupta and Premabati 2002; Kuhn et al. 1983; Lien and Siopes 1990; McNabb and King 1993; Milne et al. 1990; Schew et al. 1996; Shi and Barrel 1992; Tomasi and Mitchell 1994). In most animals, TH levels are highest during the middle of the day and lowest at night. Fluctuations also occur with age, reproductive stage, psychological state, and general health (e.g. Van den Berge et al. 1999; Stockigt 2000). As levels of THs are known to fluctuate relative to the many variables described above, static measurements (single samples) of THs and thyroid-binding protein concentrations often reported in the literature to characterize TH function cannot be reliably compared (e.g. Fowler 1989; Gagneax et al. 2001).

Tests that measure thyroxine turnover rates are also commonly done, however, and as these values reflect composite differences in TH metabolism, they may be more comparable. For example, the average half-life of T_4 is 13 hours in dogs, 16.6 hours in cats, and 6.8 *days* in humans (Kaptein et al. 1994). Some differences between animal breeds have also been demonstrated. For example, the half-life of T_4 in the beagle (a

breed that is typically diestrous) is twice that of the basenji (a breed that is typically monoestrous) (Nunez et al. 1970). Although comparisons within and between species for measured values of T_3 and T_4 obtained by different laboratory methods are problematic, it is nevertheless apparent that significant differences do occur.

Few studies have sampled TH levels frequently enough to determine the normal daily fluctuating pattern of TH production for a species. Those studies that have been done suggest marked TH daily profile differences exist between taxa (see table 2.1, section 20). Recently, for example, Gancedo et al. (1997) measured whole body T_3 and T_4 levels four times a day in larvae of three anuran species with different phylogenetic origins, behaviours and ecological habits (*Rana perezi, Xenopus laevis, Bufo calamita*). They found that daily profiles of THs differed significantly among species and during ontogenic development at two significant stages for all three taxa. Such profiles have since been shown to differ in another frog species (Wright, Duffy et al. 2003).

A similar situation has also been demonstrated in birds, where the pattern of post-hatching rise in TH levels differs significantly between neonates of species that produce rapidly-maturing (*precocious*) young, such as the European starling (*Sturnus vulgaris*), and those with slowly-maturing (*altricial*) young, such as the Japanese quail (*Coturnix japonica*), the bluenecked ostrich (*Struthio camelus)* and the emu (*Dromaius novaehollandiae*) (Blache et al. 2001; Härlid and Arnason 1999; Schew et al. 1996).

The pulsatile pattern of TH secretion appears to exert a strong influence throughout the endocrine system. While all hormones are secreted in a pulsatile fashion (Chadwick and Goode 2000; Scanlon and Toft 2000; Stockigt 2000), it is apparent from detailed study that at least some of these rhythms are not independent generated or

maintained. For example, in rats and birds (and also in humans, although perhaps to a lesser extent), THs have been demonstrated to be necessary for generating the pulsatile production of GHRH (*growth hormone releasing hormone*) by the hypothalamus (Anderson et al. 2000; Hadley 2000; Harvey 1990; Yen 2001) and are thus required for pulsatile GH release (Robinson 2000; Veldhuis 2000); in rats, THs have been demonstrated to be required for the pulsatile release of ACTH (*adrenocorticotropic hormone* or *corticotropin*) from the pituitary (Murakami et al. 1984) and are thus essential for initiating the pulsatile release of catecholamines and glucocorticoids from the adrenal gland (see references in Table 2.1, Part I, section 13).

Since the generation of virtually all pituitary and steroid hormones (from their respective endocrine-producing organs, including the gonads and the liver) have been shown to be dependent on THs, at one or more stages (see Figure 2.1 and Table 2.1), I suggest that rhythms of THs (see Table 2.1, Part I, sections 20-23) may be the pacemaker that drives or augments pulsatility in the others (see Table 2.1, Part I, sections 08, 09, 10, 14, 15, 16, 17, 18, 19). While this remains to be conclusively demonstrated, the circumstantial evidence that such a central control mechanism not only exists but has substantial biological and evolutionary significance is compelling.

It has long been suggested that THs are the biochemical agent responsible for coordinating the body's total adaptive response to both short-term (daily) and long-term (seasonal) changes in environmental conditions (Hadley 2000), allowing fully coordinated (and often rapid) modulation in such traits as timing of reproductive function and increased metabolism in response to changes in light and temperature. As a consequence, recent evidence that TH-release is distinctly pulsatile in nature and that TH

rhythms vary according to both extrinsic and extrinsic factors, suggests to me the distinct possibility that shifts in timing and intensity of TH pulses (i.e. changing *thyroid hormone rhythms, or* THRs) are the biological mechanism through which individual short-term adaptation is achieved in vertebrates. The newly-documented dose-dependent role for THs in controlling ontogenic growth and development at the molecular level (e.g. Garcia-Segura and McCarthy 2004; Lavado-Autric et al. 2003; Zoeller 2003) suggests further that THRs may also be the biological mechanism responsible for allowing vertebrates to change permanently in response to changing environmental conditions over evolutionary time.

In short, I contend that THRs are strongly implicated in the mechanism that drives evolutionary change in vertebrates not only because of the crucial role of THs in embryonic, foetal and post-natal growth and development (via effects on regulatory genes and non-genomic cellular processes), but because THs are the only known factor demonstrated so far to link (through hormonal interactions and inter-dependence) the morphological, reproductive, and behavioural traits known to change in coordinated fashion over evolutionary time.

Table 2.1, part I. Summary of supporting evidence for mechanisms or actions relating to hormone pulsatility as described or inferred in Figure 2.1 and Appendix B, Figure 2.

ACTION	DESCRIPTION (* suggests a molecular mechanism)	ANIMAL MODEL	REFERENCE
01 Retinal &/or neural stimulation & inhibition of pineal gland	**a**-light, dark & electrical stimulation of pineal elicit a hormonal response; **b**-pineal directly responsive to light;	birds; birds;	Korf 1994 (review), Meddle & Follet 1997; Yoshimura et al. 2003;
02 Direct connection from the retina to the SCN (*suprachiasmatic nuclei*) of the hypothalamus	**a**-discovery of a direct neural pathway (*retinohypothalamic tract*, RHT) connecting the retina to the SCN; **b**-signals from the retina to the SCN can be relayed to the pineal (e.g. to suppress melatonin release) or to the adrenal cortex (to suppress corticosterone secretion); ***c**-relay of retinal message from RHT to SCN neuron requires transport of Ca^{2+} into the cell in order for clock gene transcription to proceed (T_3 enhances Ca^{2+} transport, see 34 a, b)	mammals; rat; model of proposed mechanism;	Reppert & Weaver 2002 (review); Scheer et al. 2001 (review); Reppert & Weaver 2002: Figure 4 (review);
03 Origins & characteristics of hormone pulsatility & rhythmicity	***a**-individual clock genes in the SCN show rhythmic transcription; **b**-neurons in the SCN are active in the fetus by 75 days gestation; **c**-neurons in the SCN display rhythmic firing early in development & rhythmic expression of neurons requires Ca^{2+}; ***d**-SCN firing patterns appear to decrease in amplitude with age; **e**-evidence from many studies suggests that hormone secretion pathologies usually result from rhythm irregularities;	mouse; foetal sheep; rat; rat; mammals;	Reppert & Weaver 2002 (review); Breen et al. 1996; Shibata & Moore 1988; Satinoff et al. 1993; Pincus 2000 (review), Copinschi et al. 2000 (review);
04 TH & the *mediobasal hypothalamus* (MBH) region (which includes the SCN)	***a**-light & electric shock induce *Dio2* gene (in glial cells) to produce DII enzyme that converts local T_4 to T_3; ***b**-glial cells shown to express DII; ***c**-glial cells integrate signals emanating from neurons & other glial cells, including hormones like T_3; ***d**-norepinephrine (a catecholamine) increases T_4 to T_3 conversion via DII;	quail; mammals; vertebrates; rat, hamster, mouse;	Yoshimura et al. 2003; Bernal 2002 (review); Garcia-Segura & McCarthy 2004 (review); Silva 2000 (review);
05 Pulsatile release of melatonin from the pineal	**a**-evidence of daily rhythmic secretion of melatonin in a number of species;	rat, frog; 2 species of seals;	Haisenleder et al. 1992, Wright 2002, Aarseth et al. 2003;
06 Pulsatile release of TRH (*thyrotropin-releasing hormone*) from the hypothalamus	**a**-TRH pulsatile release generates pulsatile secretion of PRL & TSH; **b**-electrical stimulation of MBH increases plasma TRH levels **c**-TRH pulsatile release generates pulsatile secretion of TSH	rat; rat; human;	Haisenleder et al. 1992; Hadley 2000: 117 (review); Scanlon & Toft 2000 (review); Greenspan et al. 1986;

Table 2.1, part I (continued).

ACTION	DESCRIPTION (* suggests a molecular mechanism)	ANIMAL MODEL	REFERENCE
07 Alternate roles for TRH, in addition to stimulating pituitary to release TSH	**a**-TRH released from hypothalamus in response to neurotransmitters regulates processes in the CNS itself (neurotransmitters are synthesized within the axonal endings of neurons); **b**- in certain circumstances, GH is released from the pituitary in response to TRH;	vertebrates; all vertebrates;	Hadley 2000: 117 (review); Harvey 1990 (review);
08 Pulsatile release of GHRH (*growth hormone releasing hormone*) from the hypothalamus	**a**-blockade of GHRH interrupts GH pulsatility & GHRH stimulates GH cell proliferation & secretion in the pituitary via *adenyl cyclase* pathway;	rat, human;	Robinson 2000 (review);
09 Pulsatile release of GnRH (*gonadotropin-releasing hormone*, aka LH/FSH-RH) from the hypothalamus	**a**- GnRH pulsatility shown; **b**- GH pulse amplitude declines with age correlated with GnRH release; ***c**-rhythmic pattern of GnRH gene expression in GnRH neurons; **d**-differences in pattern of GnRH pulses can affect LH/FSH expression;	rhesus monkey; human; rat GnRH- secreting cell line (GT1-7's); rat;	Hadley 2000: 119 (review); Veldhuis et al. 2001; Gillespie et al. 2003; Haisenleder et al. 1992;
10 Pulsatile release of CRH (*corticotropin-releasing hormone*) from hypothalamus	***a**-*crh* gene transcription shows a pronounced rhythm that appears controlled by SCN;	rat;	Watts et al. 2004;
11 Alternate role for CRH in non-mammalian taxa is to stimulate TSH release (& thus thyroid hormone, TH) from the pituitary	**a**-CRH stimulates TH release, controls spontaneous & environmentally-induced metamorphosis; ***b**-CRH acts directly on TSH-producing pituitary cells (*thyrotrophs*) via binding to CRH-R2 (receptor);	birds, fish, amphibians, reptiles; chicken;	Denver 1999 (review); De Groef et al. 2003;
12 Pulsatile release of ACTH (*adrenocorticotropic hormone*, aka *corticotropin*) from the pituitary	**a**-rhythmic daily variations in plasma concentration of ACTH (sampled every 4 hrs); **b**-ACTH rhythms disorder with age; **c**-rhythm variations shown with sampling every 20 min;	rat; human; domestic cat;	Watts et al. 2004; Veldhuis 2000 (review); Kemppainen & Peterson 1996;
13 Pulsatile release of *glucocorticoids* (cortisol, corticosterone) from the adrenal cortex	**a**-corticosterone secretion is pulsatile & shows individual & breed variation; **b**-glucocorticoid release is pulsatile; **c**-cortisol release is pulsatile;	2 rat breeds; frog; human, domestic cat;	Windle et al. 1998, Lightman et al. 2000; Wright, Guertin et al. 2003; Veldhuis 2000 (review), Kemppainen & Peterson 1996;
14 Pulsatile release of PRL (*prolactin*) from the pituitary	**a**-pulsatile PRL gene expression in pituitary cells is selectively sensitive to the amplitude of TRH pulses;	rat;	Haisenleder et al. 1992;
15 Pulsatile release of TSH (*thyroid stimulating hormone*) from the pituitary	**a**-illness suppresses TSH pulsatility; **b**-TSHβ gene expression is selectively sensitive to the frequency of TRH pulses (slow more effective); **c**-pulse *amplitude* of TSH secretion, but not frequency, changed with age; **d**-with pulsatile TSH secretion, increases in serum TSH correlate to an increase in pulse amplitude & decrease in pulse frequency;	human; rat; human; human;	Van den Berghe et al. 1999; Haisenleder et al. 1992; Greenspan et al. 1991; Greenspan et al. 1986;

Table 2.1, part I (continued).

ACTION	DESCRIPTION (* suggests a molecular mechanism)	ANIMAL MODEL	REFERENCE
16 Pulsatile release of ACTH is TH-dependent	**a**-T_4 & T_3 restore ACTH rhythms abolished by thyroidectomy (- THs);	rat;	Murakami et al. 1984;
17 Pulsatile release of LH/FSH (gonadotropins) from the pituitary	**a**-LH & FSH release is pulsatile & becomes irregular with age; ***b**-an endogenous pulse-generator is present in the LHRH neuron;	human, sheep; rhesus monkey;	Veldhuis 2000 (review); Terasawa 2001;
18 Pulsatile release of α-MSH from pituitary	**a**-αMSH shown to be pulsatile with sampling every 20 min;	domestic cat;	Kemppainen & Peterson 1996;
19 Pulsatile release of LH & GH from pituitary	**a**-both LH & GH pulse amplitude declines with age; **b**-GH sampled very 10 min; **c**-GH rhythm profiles of males become disorderly with age; **d**-during growth, GH pulse amplitude increases & periodicity shifts; **e**-GH profiles of ♂ & ♀ rats differ, as do ♂ & ♀ humans;	rhesus monkey, human; human; human; human; rat, human;	Woller et al. 2002, Russell-Aulet et al. 2001; Adcock et al. 1997; Veldhuis 2000 (review); Ogilvy-Stuart & Shalet 1992; Robinson 2000 (review);
20 Pulsatile release of THs from thyroid gland	**a**-T_3/T_4 sampled every 15 min daily; **b**-T_4 daily rhythm changed seasonally; **c**-T_4 daily rhythm changed during development distinctly for each; **d**-T_4 sampled every 4 hrs daily; **e**-daily variation in circulating concentrations of T4 reflects short-term pulsatile & diurnal variation; **d**-T_3 sampled every 4 hrs daily; **e**-T_3/T_4 sampled every 4 hrs daily; **f**-T_3/T_4 sampled every 1 hr daily; **g**-T_4 sampled every 20 min daily & showed individual rhythm variation; **h**-T_3/T_4 sampled every 4 hrs daily; **i**-T_3/T_4 sampled every 20 min daily; **j**-T_3/T_4 sampled every 4 hrs daily;	cow; frog; 3 genera of amphibian; frog; human; rat; horse; fish; domestic cat; chicken; human; rat;	Bitman et al. 1994; Gancedo et al. 1996, Kuhn et al. 1983; Gancedo et al. 1997; Wright, Duffy et al. 2003; Stockigt 2000 (review); Zandieh Doulabi et al. 2004; Duckett et al. 1989; Gomez et al. 1997; Kemppainen & Peterson 1996; Cogburn & Freeman 1987; Lucke et al. 1977; Campos-Barros et al. 1997;
21 Pulsatile activity of D-II (catalyzes T_4 to T_3)	**a**-D-II sampled every 4 hrs;	rat;	Campos-Barros et al. 1997;
22 Pulsatile activity of TH-dependent genes	**a**-rhythmic expression reported for: cholesterol 7-α-hydroxylase, phosphoenolpyruvate carboxykinase, glucose-6-phospate dehydrogenase, glutamine synthetase, Spot 14 (liver); **b**-rhythmic expression shown for thyroid-receptors (TRα1 & TRα2) & mRNA expression of *c-erb Aα* genes;	rat; rat;	Zandieh Doulabi et al. 2004 (review); Zandieh Doulabi et al. 2004;
23 Rhythms of THs are influenced but not directly controlled by circadian rhythms of melatonin	**a**-effects of melatonin on T_3 & T_4 depends on season, dose & time/day; **b**-rhythms of T_4 change during ontogeny independent of melatonin changes due to light/dark;	fish; frog;	Gupta & Premabati 2002; Wright 2002;

Additional abbreviations: LH, *Luteinizing hormone* (*lutropin*); FSH, *Follicle stimulating hormone* (*follitropin*); LHRH, *Luteinizing hormone-releasing hormone*; GH, *Growth hormone* (aka STH, *Somatotropin*); IGFs, *Insulin-like growth factor-I*; α-MSH, *Alpha-melanocyte stimulating hormone* (*α-melantropin*); T3, *Triiodothyronine*; T4, *Thyroxine*; DII, *$T_4$5'-deiodinase type II*; CNS, *Central nervous system*. **Note:** Citations designated as *review*, e.g. Hadley 2000 (review), indicate the evidence listed is secondary (i.e. contains a reference to previous work of the authors or other researchers), while other citations refer to original research.

Table 2.1, part II. Summary of supporting evidence for direct and permissive actions, and interactions of THs with other hormones, as inferred in Figure 2.1 and Appendix B Fig. 2.

ACTION	DESCRIPTION (* suggests a molecular mechanism)	ANIMAL MODEL	REFERENCE
24 Need for *thyroid hormone receptors* (TRs) to interact with tissues & activate genes (TRα1, TRα2; TRβ1, TRβ2)	***a**-*c-erb A* genes express the TR proteins required for all TH-mediated changes in amphibian metamorphosis;	amphibian;	Schreiber et al. 2001, Anderson et al. 2000 (review);
	***b**-affinity for T_3 to TRs is 10-15X higher than for T_4;	amphibian;	Huang et al. 2001 (review);
	***c**-metamorphosis involves tissue-specific conversion of T_4 to T_3 moderated by particular TR isoforms;	amphibian;	Huang et al. 2001 (review), Eliceiri & Brown 1994;
	***d**-TRβ genes up-regulated by rising T_4 levels & thus TRβ-dependent processes accelerate as T_4 levels rise;	amphibian;	Huang et al. 2001 (review), Yaoita et al. 1990;
	***e**-T_3 concentrations regulate alternative spicing of the TRα pre-mRNA produced by the *c-erbAα* gene & thus control the ratios of TRα-1 & TRα-2 alternate forms of TRα;	human HepG2 cells;	Timmer et al. 2003, Flamant & Samarut 2003 (review);
	***f**-alternate forms of TRβ are also produced by alternative splicing ;	amphibian;	Yaoita et al. 1990;
25 Need for THs plus GHRH to elicit release of GH from the pituitary	**a**-THs required for GHRH release;	human;	Hadley 2000:321 (review);
	b-THs plus adrenal glucocorticoids stimulate GH release from pituitary;	birds;	Harvey 1990 (review);
	c-GH inhibits TSH secretion;	vertebrates;	Hadley 2000:118 (review);
	***d**-T_3 stimulates transcription of GH mRNA & GH synthesis;	rat;	Yen 2001 (review), Anderson et al. 2000 (review);
	e-*T_3 stimulates GH release but has variable effects on transcription;	human;	Yen 2001 (review);
26 Need for THs plus PRL-RH to elicit release of PRL from the pituitary	**a**-TH & PRL concentrations rise together, TH required for PRL?;	turkey, human, cattle, sheep;	Hadley 2000:321 (review); Lien & Siopes 1990;
	b-TRH stimulates release of PRL	rat;	Hadley 2000: 117;
27 Need for THs plus ACTH to elicit release of adrenal hormones from the adrenal	***a**-T_3 required for steroidogenesis of adrenal glucocorticoids (e.g. cortisol) & catecholamines (e.g. adrenaline) from cholesterol substrates;	human foetus, sheep foetus, mouse;	Simonian 1986, Fowder et al. 2001, Manna et al. 1999 (review), Jefcoate 2002 (review);
28 Need for THs plus gonadotropin (LH/FSH) to elicit release of reproductive hormones from the gonads (*oestradiol* & testosterone) [the liver converts *oestradiol* to *oestrogen*]	**a**-T_3 + LH stimulate androgen release from Leydig cells of testes;	goat;	Jana & Bhattachacharya 1994;
	b-T_4 + FSH/LH increased oestradiol & progesterone secretion in ovarian granulosa cells;	human;	Wakim et al. 1995;
	***c**-T_3 increased cholesterol convers-ion (pregnenolone to progesterone step) in ovarian follicle cells;	fish;	Bhattachacharya et al. 1996;
	d-T_3 stimulated progesterone release in ovarian granulosa cells;	rat;	Bandyopadhyay et al. 1996;
	e-T_3 alters conversion of testosterone to oestradial in granulosa cells of the ovarian corpus luteum;	human;	Southren et al. 1974;
	***f**- T_3 + LH increased testosterone secretion in Leydig cells of testes;	mouse;	Manna et al. 1999;
29 Need for THs plus GH to elicit release of IGF-I	**a**-THs increase the secretion of GH, which subsequently increases GH-stimulated IGF-I synthesis;	rat, human;	Wolf et al. 1989, Weiss & Refetoff 1996;

Table 2.1, part II (continued).

ACTION	DESCRIPTION (* suggests a molecular mechanism)	ANIMAL MODEL	REFERENCE
30 Need for THs plus GH or IGF-I to elicit action on target tissues & cells	***a**-TRα in bone cells & its interaction with THs required for normal bone growth & development (*not* TRβ) (in bone, T_3 regulates chondrogenesis, matrix synthesis, angiogenesis & mineralization);	human;	Bassett & Williams 2003;
	b-T_4 + GH required to restore function in epiphyseal growth plate in TH-deprived subjects;	rat;	Lewinson et al. 1989;
	c-THs + GH required for stimulation of ODC (*ornithine decarboxylase* – for polyamine biosynthesis & thus regulation of nucleic acid & protein biosynthesis) in the brain vs. GH- independent action in other tissues;	mammal;	Hadley 2000:323 (review);
	***d**- T_3 + IGF-I may be needed for optimum action of T_3 for myosin expression in muscle tissues;	chicken;	Gardahaut et al. 1992;
31 Need for THs plus adrenal hormones (glucocorticoids & catecholamines) to act on target tissues	**a**-pulmonary & renal enzyme synthesis need both T_3 & cortisol;	foetal sheep (late);	Forhead & Fowden 2002;
	***b**-the effects of catecholamines on adrenergic receptors are enhanced significantly by T_3 (via cAMP production;	rat, human;	Silva 2000;
32 Need for THs plus *oestradiol* (E_2) to act on target tissues	***a**-E_2 + IGF-I + T_3 needed for bone remodelling (DII enzymes in the chondrocytes convert T_4 to T_3);	human;	Bassett & Williams 2003;
33 Need for THs plus PRL to act on target tissues	**a**-PRL + THs regulate mammary gland development;	mouse;	Hadley 2000:321 (review);
34 Need for THs in general metabolism	**a**-carbohydrates or mixed diet intake increase conversion of T_4 to T_3;	human;	Hadley 2000 (review);
	***b**-the *SREBP-2* gene (*sterol regulatory element-binding protein-2*) needed for uptake of LDL cholesterol (*low density lipoprotein*) is regulated by T_3;	mouse;	Shin & Osborne 2003;
	***c**-LDL cholesterol binding sites requiring T_3 are found in highest concentrations in membranes of the adrenal cortex & ovariarn corpus luteum (where conversion to steroid hormones takes place);	human;	Hadley 2000:368 (review);
35 Need for THs in cell membrane activities	***a**-T_3 directly stimulates Ca^{2+}-ATPase production in cell membranes;	human red blood cells (erythrocytes);	Davis at al. 1989 (review),
	***b**-T_3 (in < 1 min.) & T_4 increase cytoplasmic Ca^{2+} concentration in cells in a dose-dependent manner via enhanced influx of extracellular Ca^{2+} & then, T_3 increases adenylate cyclase activity in cell membranes (thus driving ATP to cAMP);	rat thyrmus cells (thymocytes);	Segal & Ingbar 1989;
	***c**-T_3 induces synthesis of Na^+/K^+-ATPase needed to activate Na^+ pump;	general;	Hadley 2000: 323 (review);

Table 2.1, part II (continued).

ACTION	DESCRIPTION (* suggests a molecular mechanism)	ANIMAL MODEL	REFERENCE
36 Need for THs to activate functions of the mitochondria	**a**-T_3 increases O^2 consumption in mitochondria; ***b**-T_3 increases O^2 consumption & oxidative phosphorylation in as little as 2 minutes; ***c**-T_3 required to make the ATP that primes NA^+ pump (heat production); ***d**-T_3 increases mitochondrial DNA transcription via mtTR (P43) binding to T_3RE in the mtD-loop which makes more mitochrondria; ***e**-T_3 affects Ca^{2+} signalling within minutes; ***f**-*c-erb Aα* encodes the gene for a T_3 receptor (p43) found in cell nucleus & mitochondria, allowing coordinated expression of mitochondrial genes & the nuclear genes for proteins used in mitochondrial processes, thus coordinating mtDNA functions;	chicken; vertebrates (human, rat, mouse, rabbit, chicken, amphibian);	Klandorf & Sharp 1985; Wrutniak et al. 2001 (review); Wrutniak et al. 2001 (review); Wrutniak et al. 2001 (review); Wrutniak et al. 2001 (review); Wrutniak et al. 2001 (review);
37 Need for THs needed in placental development	**a**-placental trophoblasts have a high binding capacity for T_3, suggesting their growth & development may be T_3-dependent;	human;	Kilby et al. 1998;
38 Need for THs in embryonic cell migration & differentiation	**a**-THs required for tooth eruption; **b**-THs affect tooth enamel formation; **c**-THs required for CNS development (including brain, eyes), skeletal growth & maturation; **d**- T_3 required for myosin expression (regulation of embryonic MHC gene isoforms) in muscle development; ***e**-expression of fetal brain genes that affect proliferation & differentiation of cortical neurons are responsive to T_4 from mother (*neuroendocrine-specific protein* (NSP) & *Oct-1*);	human; human; human, vertebrates; vertebrates; foetal rat;	Pirinen 1995; Noren & Alm 1983; Chan & Kilby 2000 (review), Bernal 2002 (review), Schriebman et al. 1993 (review); Gardahaut et al. 1992 (review); Lavado-Autric et al. 2003;
39 Need for THs in embryonic & neonatal brain development	***a**-T_3 required for differentiation of CNS glial cells and myelin formation; ***b**-mild maternal T_4 deficiency affects normal neuron migration & thus cytoarchitecture in the fetal brain (especially in the somatosensory cortex & hippocampus); **c**-absence of T_4 affects normal growth & development in the cerebral cortex, visual & auditory cortex, hippocampus, cerebellum; ***d**-TRα-1 present throughout the brain from early fetal stages onward, while TRβ-1 rises sharply (40-fold) ca. 10 days after birth coincident with a neonatal surge in T_3;	vertebrates; foetal rat; mammal; mammal, chicken, amphibian;	Garcia-Segura & McCarthy 2004 (review); Lavado-Autric et al. 2003, Zoeller 2003; Yen 2001 (review); Yen 2001 (review);

Table 2.1, part II (continued).

ACTION	DESCRIPTION (* suggests a molecular mechanism)	ANIMAL MODEL	REFERENCE
39 Need for THs in embryonic & neonatal brain development (con't)	***e**-TR knock-out models indicate that other TR isoforms can substitute or compensate for a deficient or defective receptor, thus hypothyroid-ism (e.g. iodine deficiency) is much more devastating to development than lack of receptors;	mouse;	Kőhrle 2000 (review), Flamant & Samarut 2003 (review);
	***f**-TH regulates the genes for *myelin basic protein* (MBP), *brain-derived neutropic factor, glutamine synthase, protein kinase C, substrate RC3/neurogranin, prostaglandin D2 synthase, hairless* (a potential transcription factor), *neural cell adhesion molecule, tenascin* (matrix protein) & proteins needed for neuronal migration;	mammal;	Yen 2001 (review);
	***g**-T_3 regulates genes for *Purkinje cell-specific protein-2* (PCP-2), *Rhes*, neurotrophin-3, *Reeling, Rev-ErbAα*;	rat;	Manzano et al. 2003;
	***h**-TRβ knock-out models show specific developmental anomalies of the inner ear (cochlea) that explain the deafness seen in early onset hypothyroidism;	mouse;	Rűsch et al. 2001, Flamant & Samarut 2003 (review);
	***i**-THs stimulate synthesis of *nerve growth factor* (NGF), which induces dendritogenesis & regeneration of sympathetic neurons, as well as accelerating axonal regeneration in the cerebellum;	rat;	Hadley 2000:321 (review);
	***j**-full length *epidermal growth factor receptor* (EGF-R) mRNA is up-regulated rapidly by T_3;	rat, human	Satoh & Sairenji 1997, Chan & Kilby 2000 (review);
	***k**-the same receptor (EGF-R) mediates cell proliferation of both EGF & *transforming growth factor α* (TGFα);	human;	Raja et al. 199;

Additional abbreviations: LH, *Luteinizing hormone* (*lutropin*, a gonadotropin); FSH, *Follicle stimulating hormone* (*follitropin*, a gonadotropin); GH, *Growth hormone* (aka STH, *Somatotropin*); IGFs, *Insulin-like growth factor-I*; PRL-RH, *Prolactin-releasing hormone*; Ca^{2+}, *Calcium ion*; ATP, *adenosine triphoshate*; cAMP, *cyclic adenosine monophosphate* ("second messenger of hormone action"); O^2, *oxygen*; ATPase, *adenosine triphosphatase*; Na^+, *Sodium ion*; K^+, *potassium ion*; mtDNA, *mitochondrial DNA*; nDNA, *nuclear DNA*; mtTR, *mitochondrial thyroid receptor*, mtD-loop, *Mitochondrial displacement loop* (the site on the genome where replication begins); T_3RE, *T_3 responsive element*; MHC, *Myosin heavy chain;* T_3, *Triiodothyronine*; T_4, *Thyroxine* (aka *tetraiodothyronine*); DII, *$T_4$5'-deiodinase type II*; CNS, *Central nervous system*. **Note:** Citations designated as *review*, e.g. Hadley 2000 (review), indicate the evidence listed is secondary (i.e. contains a reference to previous work of the authors or other researchers), while other citations refer to original research.

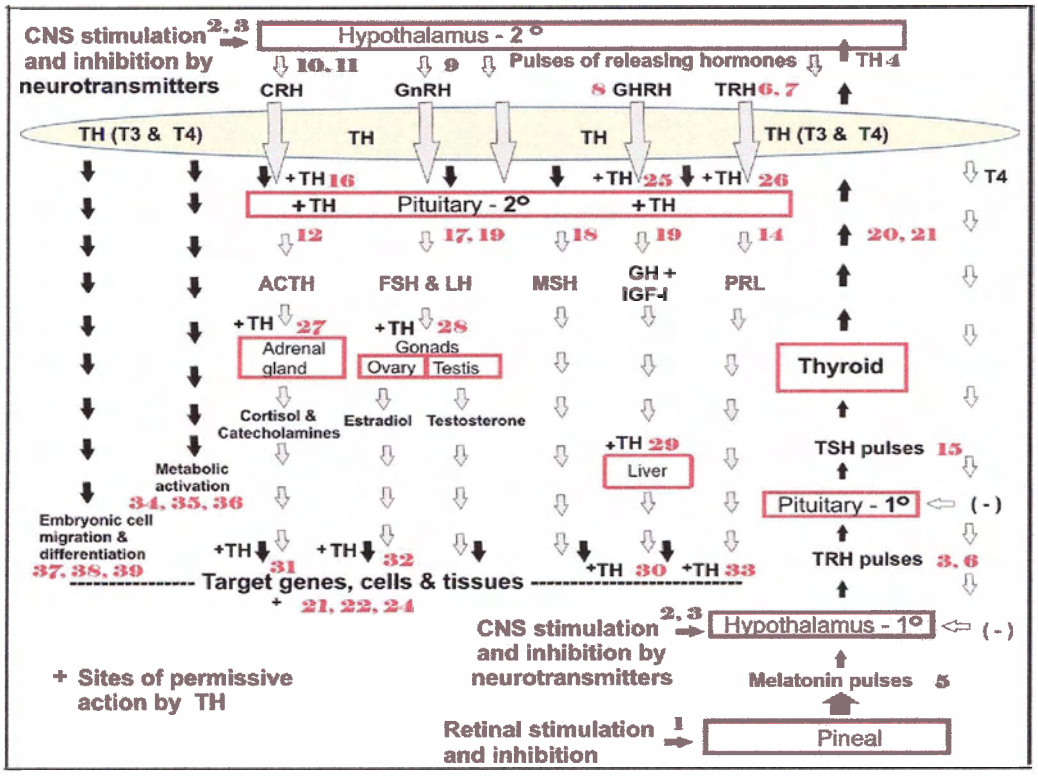

Fig. 2.1. A model I suggest more accurately depicts the role of THs on the actions and effects of pituitary hormones than do standard hormonal cascades (modified from Appendix B, Figure 2). A primary release of TH via pineal stimulation (far lower right) and/or direct hypothalamic stimulation, generates a pool of THs (top) that is permissively required (indicated by +) for both the release of hypothalamic and pituitary hormones and their subsequent affects on target genes, cells and tissues. See Table 2.1 for a list of supporting evidence for steps numbered 1-39 (with references). Abbreviations: CNS, central nervous system; TRH, thyrotropin-releasing hormone; TSH, thyroid stimulating hormone; GnRH, gonadotropin-releasing hormone; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropin (adrenal cortical stimulating hormone); FSH, follicle stimulating hormone; LH, luteinizing hormone; MSH, alpha-melanocyte stimulating hormone; GH, growth hormone (which stimulates release of insulin-like growth factor-I or IGF-I from the liver); PRL, prolactin.

2.2. Thyroid hormone and individual variation

It is my contention that pulsatile TH secretion may be the crucial underlying factor in protodomestication and other types of heterochronic speciation. Precision in timing (frequency of TH pulses) and absolute amounts of TH secreted (amplitude of TH pulses) must be critical to certain target genes, cells or organs during development, in a manner similar to the dose- and timing-dependent changes Nijhout (1999) has demonstrated for the effects of *juvenile hormone*, the substance which controls development in insects. If so, very slight individual variations in THRs within wild populations of all taxa could produce small physiological changes that are ultimately manifested as noticeable individual differences in morphological, behavioural and reproductive traits (phenotypes).

Manifestations of such individual physiological differences may be most readily apparent as variations in coat colour and size, because of the influence of THs on growth and development, and on secretion of *melanocyte stimulating hormone* (MSH), with consequent effects on hair colour, and coat length and thickness. In addition, although less immediately noticeable, individual differences in THRs almost certainly underlie the slight individual variations in timing of reproductive events (including ovulation and testes maturation) and annual moult that are recognized in virtually all mammalian taxa, as well as the individual variations in behavioural responses to stress (including levels of social dominance, territoriality, and fearfulness) that have also been documented (Banfield 1974; Fox et al. 1997; Hayes and Jenkins 1997; Parker and McKinney 1999). Provisional support for this hypothesis comes from a study on a population of young marine flatfish (aka flounder, Pleuronectiformes) following metamorphosis: Gavlik et al.

(2002) reported individual differences in TH status within the group correlated so strongly with differences in timing of "settling" behaviour into adult habitat and the growth spurt which followed that both behaviour and growth of the group could be synchronized by manipulating the thyroid status of individuals.

Individual variation in THRs are also expected to generate particular intraspecific differences within populations because of the intimate relationship shown to exist between THs, reproductive hormones and growth hormone (see also Figure 2.1 and Table 2.1). Reproductive and growth hormone have been shown to be generated in individually-unique rhythmic fashion (Buyse et al. 1990; Thompson Jr. et al. 1992; Robinson 2000; Veldhuis 2000), although the researchers responsible for these studies generally attribute little biological significance to such slight profile variance. However, I suggest that the interactions between these hormones and individual THRs probably contribute much more to individual phenotypic variation than previously thought. Considering the demonstrated inter-dependence of oestradiol, IGF-I (*insulin-like growth factor-I,* a liver protein stimulated by GH and actually responsible for most of the essential functions usually associated with growth) and THs on bone growth and remodelling (Bassett and Williams 2003; Clark and Rogol 1996; Grumbach 2000), as well as the demonstrated "booster" effect of oestrogen on both GHRH and GH/IGF-I pulsatile release (Veldhuis and Bowers 2003), these hormonal interactions may provide a partial physiological explanation for the heterochronic nature of species-specific sexual dimorphism (e.g. mustelids, Dayan and Simberloff 1994; rats, Gustafsson 1994; primates, Martin et al. 1994; German and Stewart 2002; sea lions and fur seals (Otariidae), Miller et al. 1996; the arctic fox (*Alopex lagopus*), Prestrud and Nilssen 1995): differences between the

sexes involve sex-specific differences in rates of growth and development that are consistent within each species.

However, it is also possible that in addition to the hormonal interactions described above (or perhaps instead of), THs may have a more direct impact on the development of species-specific sexual dimorphism. Although the standard paradigm of sexual differentiation presumes that sex-specific genes on chromosomes (so-called "genetic sex") determine an animal's gonad type during foetal development and that gonadal hormones are subsequently responsible for the sex-specific phenotype of all other tissues, this interpretation is now being challenged. Recently, researchers have suggested that the genetic sex of an individual may generate sex-specific differences in critical brain structures during early embryonic development (discernable well before gonads are functional) and that these sexually-distinct brain structures then control sex-appropriate development of gonads and other tissues (see reviews by Arnould et al. 2004; De Vries 2004). If this new interpretation turns out to be correct, given the critical importance of THs to embryonic neural tissue growth and differentiation that has already been demonstrated (e.g. Chan and Kilby 2000; Garcia-Segura and McCarthy 2004; Lavado-Autric et al. 2003; Zoeller 2003), it is possible that species-specific sexual dimorphism is actually a result of species-specific THRs interacting with the genetic sex of an individual during embryonic development, and that these interactions construct sex-appropriate brain structures that subsequently guide development of other sex-specific tissues.

A similar explanation may apply to other kinds of discrete within-species alternatives of physical form or *morphotypes* (Dickhoff 1993; Reilly et al. 1997), as occur in certain fish, amphibian and reptile species (e.g. male tree lizard morphs of *Urosaurus*

ornatus (Knap and Moore 1996); sockeye salmon/kokanee morphs in *Oncorhynchus nerka* (Wood and Foote 1996); and the age-specific morphs seen in metamorphic salamander species (Voss 1995; Voss and Shaffer 1997): differences between morphotypes involve morph-specific growth pattern differences that are consistent within each species. While the involvement of THs with growth and sex hormones (or with genetic sex determiners during embryonic development) does not explain why males are the larger sex in some taxa (e.g. many carnivores) while females are larger in others (e.g. birds of prey, some whales), or why particular morphotypes take one form rather than another, but if THRs are species-specific (as I maintain must be the case), it would explain the heterochronic nature of those differences and their continued species-specific expression, generation after generation.

The importance of individual differences in physiology to evolution are becoming more apparent as researchers attempt to understand what factors influence the success of populations over time, as McNamara and Houston (1996) have discussed in detail. For example, Crowder et al. (1992) found that individual physiological differences among fish larvae in their study were extremely critical to recruitment success under certain environmental conditions; larvae that survived to adulthood were not "average individuals" but represented a specific fraction of the population that possessed particular physiological phenotypes. In other words, survivors were not a random subset of the original population but a group of physiologically similar animals particularly suited to the specific set of environmental conditions associated with their early growth. Such phenomena are particularly germane to this argument (see further discussion, Section 3.1).

It is apparent that individual variation in hormone physiology needs to be studied more carefully within an evolutionary context. While phenotypic variation, generally speaking, can be said to exist because of slight mutational changes that occur randomly and continuously in genes, such genes do not necessarily have a one-to-one correspondence with specific morphological, behavioural or reproductive traits that are equally subject to natural selection. Genetic variation must be exposed, through its epigenetic effects on the biochemistry, physiology, and development of the individual before it can be subjected to natural selection (Hall 1992). All levels of expression of the genotype, including behaviour, may be subject to natural selection (West-Eberhard 1989; 2003). A multi-tiered aspect of gene expression means that selection for one phenotypic trait (at any level, such as size) can trigger a non-linear cascade of changes in associated traits (such as reproductive timing, behavioural responses to stress and coat colour differences) that may appear unrelated but are biochemically, physiologically and/or developmentally linked (Alberch 1991). As I have shown, THs control or influence many characteristics in just such a complex fashion, via direct actions on regulatory genes, cells and tissues that affect growth and development as well more indirect effects generated by interactions with pituitary and adrenal hormones, and with neurohormones of the central nervous system.

The potential existence of distinct species-specific THRs (with associated individual rhythm variants responsible for phenotypic variations in behaviour, morphology, and reproductive physiology) might explain the fact that some species within the genus *Canis*, such the wolf, *C. lupus*, display great phenotypic variability over the whole of their range (Jolicoeur 1959, 1975; Mech 1970; Young and Goldman 1944),

although the coyote, *C. latrans,* does not (Mengel 1971; Young 1951). If such phenotypic variation indeed reflects the range of differences in THRs within and between species, wolves should have a larger range of hormonal variation than coyotes, giving them more evolutionary options (see Gould's (2002:1271) discussion of differential "flexibility for future change" or *evolvability*). This may explain why the wolf has generated dog descendants several times in the last 15,000 years as an adaptive response to human-dominated habitats (see Section 3.22), while the coyote has merely expanded its range (Nowak 1979).

2.3 Thyroid hormone and coat colours

Although the size, shape and reproductive changes in biological traits associated with protodomestication (Section 1.2) have been shown to be under the control of THs, the appearance of piebaldness (black or any other solid colour marked with white) is the hardest to explain in this context. The high incidence of piebaldness in domestic animals has always been somewhat of an enigma and is usually assumed to be a consequence of deliberate selection (e.g. Clutton-Brock 1992a). Piebald markings are rare in most wild mammals, including the ancestors (or their close extant relatives) of domestic taxa, although it is the norm in a small number of groups (e.g. skunks). Such a low natural incidence of piebaldness in wild populations would make increasing the proportion of individuals with this trait (via natural or human selection) very difficult. However, the early appearance of piebaldness in the silver foxes experimentally selected for behavioural phenotypes (as discussed in Section 1.3), suggests that piebaldness could be an inevitable consequence of the heterochronic process of protodomestication. To

explain why this is so, we need a short explanation of pattern formation and pigment production.

Pigment (technically *melanin*) is produced in special cells called *melanocytes* that exist in skin, hair follicles and other body tissues; this is where the chemical conversion of *tyrosine* (derived from the ingested essential amino acid *phenylalanine*) to melanin takes place. Many different factors can affect melanocyte activity during pigment production, including hormonal influences from the pituitary (especially from MSH, or *α-melanocyte-stimulating hormone*) as well as spontaneous mutations in hormone receptors and cofactors in the follicles, resulting in different coat and skin colours (Hearing 1993; Kijas et al. 1998; Robbins et al. 1993; Searle 1968).

Patterning of colour, however, is determined during foetal development (details given here from Silvers 1979). The hypothesis that so far best explains colour pattern anomalies suggests that, during early embryonic development, seven pairs of *melanoblasts* (undifferentiated melanocyte precursors) must migrate from their origins in the central neural crest to areas in the body where pigment is required. Through controlled proliferation of these melanoblasts and their subsequent maturation to functional melanocytes, a normal pattern of pigmentation is produced; typically, colour spreads from two central areas of melanoblast concentration at the base of the tail and between the eyes, and from symmetrical patches over the hips, lumbar region, rib cage, ears, eyes and nose – 14 areas in all. Areas in between these centres that do not receive melanoblasts because of disruption of cell migration or subsequent cell proliferation remain white, resulting in a coat colour pattern that is variously spotted. For example, the piebald coat patterns in affected foxes from Belyav's selected population (similar to the

pattern of a typical Border collie) were ultimately determined to be caused by a one- to two-day delay in the migration rate of the melanoblasts from the neural crest in the embryo (Prasolova et al. 1997). Piebald colouration can vary from only a spot or two of white on a solid colour background to an all-over white that is essentially one big spot.

During development, embryonic neural crest tissue is the source of several specific cell lineages in addition to epidermal and choroidal pigment cells, including neurons and glia of the peripheral nervous system, neuroendocrine cells, and pharyngeal arch-derived tissues of the face and neck (Hosada et al. 1994). Specific mutations causing failure of neural crest precursors of epidermal melanocytes and enteric ganglion neurons to migrate properly in the embryo have been shown to result in nerve supply defects of the intestine (*aganglionic megacolon*) in humans, mice and horses that are associated with various levels of piebaldness (Bowling 1994; Geissler et al. 1988; Hosada et al. 1994; Lamoreux and Russell 1979; Witte 1990). Similarly, inner ear sensory cells also originate in the neural crest (Cowling et al. 1994), explaining the correlation of some white or nearly-white animals with hearing loss (Belyaev et al. 1981; Silver 1979) as a failure of neural crest precursors to migrate appropriately.

Both T_4 and retinoic acid (a vitamin A derivative) have been identified as essential to the orderly movement of cells out of the neural crest during early development (Barres et al. 1994; Bennett 1991; Cowling et al. 1994; Hosoda et al. 1994; Pavan and Tilghman 1994; Pavan et al. 1995). The T_4 needed for neural crest cell migration is required by the embryo very early in development and, in mammals (including humans), the maternal thyroid gland has been shown to be the source of the T_4 utilized (Barres et al. 1994; Chan and Kilby 2000; Pickard et al. 1993; Porterfield 2000; Stephanou and Handwerger 1995).

The foetal thyroid gland is not functional until fairly late in development (about 18-20 days for the rat, about 50 days for the ewe (Morreale de Escobar et al. 1985), and 10-12 weeks in humans (Pickard et al. 1993)), at which time it begins to augment rather than replace the maternal contribution (Chan and Kilby 2000). Consequently, although a direct correlation between disruption of thyroxine production and piebaldness has not yet been demonstrated, the circumstantial evidence in favour of such a relationship is very strong.

Piebaldness has been a very useful experimental indicator of early developmental errors. There are at least three genes in which mutations have been shown to cause disruption of the normal migration of melanocytes during early development. Specific mutations in each of these genes result in a piebald pattern of pigmentation combined with various neurological disorders (Bennett 1991). The *piebald* (S) gene has been found to act earlier in foetal development than either *dominant white-spotting* (W) or *steel* (Sl) genes (Geissler et al. 1988; Pavan and Tilghman 1994; Pavan et al. 1995; Witte 1990;) and thus tends to produce distinctly different piebald patterns than the others.

Mice with mutations in white-spotting genes (S/W/Sl) have been used in an attempt to unravel the development of piebald phenotypes. However, these experimental mutants may not possess the same genotypes as piebald wild animals or most piebald domesticates. For example, the dominantly inherited extreme piebaldness (Hosada et al. 1994) demonstrated by most "black-eyed white" domestic and wild animals (e.g. many sheep breeds, Park White cattle, the polar bear and mountain goat, *Ovis canadensis*) (Alderson 1978; Searle 1968) is not correlated with the lethal *megacolon* or the deafness defects associated with severe forms of *dominant white-spotting* seen in experimental

animals (Belyaev et al. 1975, 1981; Geissler et al. 1988). Obviously, piebald phenotypes can result from several distinct genetic abnormalities.

Unfortunately, no empirical studies appear to have been done on the specific types of piebaldness demonstrated by wild taxa. Similarly, studies on the inheritance patterns of piebald spotting in domestic animals (Bowling 1994; Olson 1981; Searle 1968; Woolf 1995) have not added much to our understanding of the underlying mechanisms involved (that is, we may be able to predict how the pattern will be transmitted in various crosses, but we do not know exactly which gene product is actually producing the patterns). However, as piebald coat colour patterns reflect developmental variants, their consistent appearance in domesticates suggests that piebaldness may be a direct consequence of the heterochronic changes seen in protodomestication and may be in certain wild taxa as well.

While the incidence of piebald coat colours has increased dramatically in natural domestic taxa, the actual colour of pigment produced in melanocytes also appears to have been affected by protodomestication changes. These changes have resulted in domestic animals that commonly possess non-*agouti* coat colours in shades of solid red, yellow, brown and black (*agouti* refers to the alternating bands of colour found in the individual hairs of many wild mammals, giving a grizzled appearance). Since melanin production is governed in part by hormonal influences from the pituitary, via MSH release (in addition to the plethora of chemical interactions necessary for the manufacture of pigment within the melanocyte), it appears that this colour change could have occurred as a result of protodomestication. The inheritance patterns of many of the genes influencing coat colour have been studied extensively (Keeler 1975; Little 1958; Shackleford 1984; Silvers 1979), although the underlying molecular mechanisms have been demonstrated

for only a few.

One of the colour-producing genes that is expressed in the post-natal environment, *extension (E)*, has been found to encode the receptor (MC1R) for α-*melanocyte stimulating hormone* or MSH (Burchill et al. 1993). MSH induces the synthesis of *tyrosinase*, the enzyme that mediates the production of melanin in the follicle. High levels of tyrosinase are required for the synthesis of *eumelanin* (brown/black pigments), leaving *phaeomelanin* (yellow/red) as the "default" pigment produced in the presence of low tyrosinase levels. As THs initiate MSH secretion (Hadley 2000), levels of available THs must influence coat and skin colour production. Indeed, in deer mice, differences in TH levels have been noted between *agouti* and non-*agouti* coloured individuals (Lapseritis and Hayssen 2001). In addition, early research on the *agouti* (*A*) gene (Bultman et al. 1992; Robbins et al. 1993; Siracus et al. 1995) indicated it might encode the ligand for the *extension* gene mentioned above (now confirmed as a protein that inhibits MC1R activation; Schmutz et al. 2003), meaning that this gene is associated with the hormonal response of melanocytes to MSH. Pavan et al. (1995) suggest that *piebald* (*S*) may also be a modifier of the *Extension* gene.

Although hormonal influences can generate non-*agouti* pigmentation, solid colours can also be produced in other ways. Spontaneous mutations within the *extension* and *agouti* genes themselves are responsible for producing some red, yellow, brown and black coat colours (Robbins et al. 1993; Siracus et al. 1995; Schmutz et al. 2003). In other words, defective or hyperactive receptors, receptor ligands and/or promoters necessary for triggering melanin synthesis within the hair follicle can produce similar (or identical) phenotypes to those produced by high or low levels of available of MSH.

The prevalence of these non-*agouti* solid colours in all domestic animals (Alderson 1978; Searle 1968), especially when they do not occur in wild populations of their ancestors or close extant relatives (or are extremely rare), suggests that a common consequence of domestication is a change in the relationship between THs and melanocytes. This relationship could have changed either as a result of mutations in the target loci (hormone receptors and their cofactors in the hair follicle) or due to shifts in the timely availability or concentrations of THs. I suggest that the hormonal relationship probably changed first as a consequence of protodomestication and that over time, mutations at some target loci occurred and were perpetuated by artificial selection. Thus in modern domestic animals, both types of non-*agouti* colouration probably exist; this perhaps explains the lack of receptor involvement (of either *agouti* or *extension)* in "dominant black" colouration in some dogs (Kerns et al. 2003), despite the confirmation of changes to the *extension* receptor locus in the similar phenotype, "melanistic black mask" (Schmutz et al. 2003). Surprisingly, humans and other primates, like domesticates, also lack typical mammalian *agouti* colouration (Robins 1991; Searle 1968).

In contrast to hormonally controlled pigmentation, mutations of genes that operate chemically within the melanocyte or hair follicle during synthesis of melanin (such as *albino*) are not implicated in domestication changes. Albino phenotypes occur occasionally in many domestic and wild taxa (Hearing 1993; Little 1958). Some of these phenotypes have been perpetuated in domesticates through artificial selection and in some wild populations through genetic drift. However, albino phenotypes are not a consistent ubiquitous trait of domestic taxa.

2.4 Thyroid hormone and behaviour

In addition to controlling the development of specific morphological traits, THs also affect behaviour. Principally, THs mediate behavioural responses to stress and stimuli that are influenced by sympathetic adrenal gland function. This is due to the fact that production of adrenal *catecholamines* (*epinephrine, norepinephrine* and *dopamine*) is controlled by adrenergic receptors and activity of these receptors is strongly affected by levels of available THs (Hadley 2000; Goldman et al. 1993; Fowden et al. 2001; Manna et al. 1999; Schreibman et al. 1993). Thus behaviour relating to an animal's stress response is fundamentally under TH control: just as THRs show individual and intraspecific variation, so do stress responses (Boissy 1995). In rats and wolves, certain individuals have been found to produce less adrenalin or react less when exposed to stress and stimuli than others (Fox 1978; Hemmer 1990; Henshaw et al. 1979; Naumenko 1973). Breed differences in biochemical stress responses of dog morphotypes have also been demonstrated (Arons and Shoemaker 1992; Stockard 1941).

Hadley (1984:337) has defined stress as "the state resulting from events (stressors) of external or internal origin, real or imagined, that tend to affect the homeostatic state,... any condition tending to elevate plasma catecholamine [i.e. adrenal hormone] levels in response to exogenous or endogenous stimuli." Therefore, the ability to respond to the varied exogenous stresses which are fundamental to survival should be affected by an individual's particular THR. Individual differences in THRs could therefore account in part for individual differences in stress responses and in social dominance behaviour evident within species, given that the inter-personal social pressures that affect communal dwelling animals represent particular kinds of stress that generate particular behavioural

responses (see discussions in Boissy 1995; Fox et al. 1997; Hardy et al. 2002; Morgan et al. 2000).

2.5 Thyroid hormones and development

As discussed in Section 2.3 in relation to piebald phenotypes, THs are known to be required for normal embryonic development at all stages - supplied by the maternal system either directly (for mammals) or via reserves stored in egg yolk (for non-placental vertebrates). Embryonic neural crest tissue is the source of several essential cell lineages that are dependent on retinoid acid and T_4 for properly timed migration, proliferation and maturation, including epidermal and choroidal pigment cells, neurons and glia of the peripheral nervous system, neuroendocrine and inner ear sensory cells, and pharyngeal arch-derived tissues of the face and neck (Barres et al. 1994; Cowling et al. 1994; Hosada et al. 1994; Pavan et al. 1995). In the developing digestive system, THs are known to be responsible for the differentiation of the epithelial lining of the small intestine (where nutrient absorption occurs) and to effect the timing of tooth eruption (Pirinen 1995) and tooth enamel formation (Noren and Alm 1983). T_3, has been shown to be critical for the expression of various myosin isoforms during embryonic, neonatal and adult muscle fibre formation (Gardahaut et al. 1992). Both T_4 and T_3 have been identified as essential, in animal models such as the rat, for oligodendroctye differentiation, axonal myelination, dendritic and axonal growth, neurotransmitter regulation and synaptogenesis in the central nervous system (Chan and Kilby 2000; Dubois-Dalcq and Murray, 2000; Garcia-Segura and McCarthy 2004; Lavado-Autric et al. 2003; Park et al. 2001; Smallridge and Ladenson 2001). In short, a wide range of the myriad small steps that constitute the

process of embryonic development are known to be controlled by THs (Yen 2001).

This early direct role for THs is not the only aspect of its effect on development, because of the way THs interact with the production and actions of other hormones. Growth and sex hormones are also required for development; for example, T_3 has been shown to be an essential regulator, along with GH and oestrogen, of linear growth via its direct affects on the cartilaginous growth plates of long bones (Bassett and Williams 2003). GH has been shown to be dependent on THs for its expression and timing of reproduction is strongly correlated with TH levels (see Figure 2.1; the discussion of sexual dimorphism in Section 2.2; Basset and Williams 2003; Grumlach 2000; Yoshimura et al. 2003).

In non-placental vertebrates, all required hormones (as well as vitamins, essential fatty acids, steroid hormones and growth factors, such as IGF-I) are present in the yolk (De Pablo 1993; Elinson 1987; Rol'nik 1970; Speake et al. 2002). In fish, THs are incorporated during the initial formation of yolk through the process of *vitellogenesis* (Jobling 1995). The exact amounts of essential hormones and other nutrients incorporated must be controlled by the maternal system (as shown by Wilson and McNabb (1997), who found that T_4 concentration of yolk varied with the TH status of the hen), putting non-mammalian vertebrates in a similar position as placental mammals in regards to achieving maternal control over early embryonic development (Figure 2.2).

In mammals, not all hormones can flow through to the foetus directly from the maternal system. Chan and Kilby (2000) recently summarized current knowledge regarding THs and development in humans and notes that the placental barrier is impermeable to TSH and growth hormones (GH). However, TRH, iodide (iodine

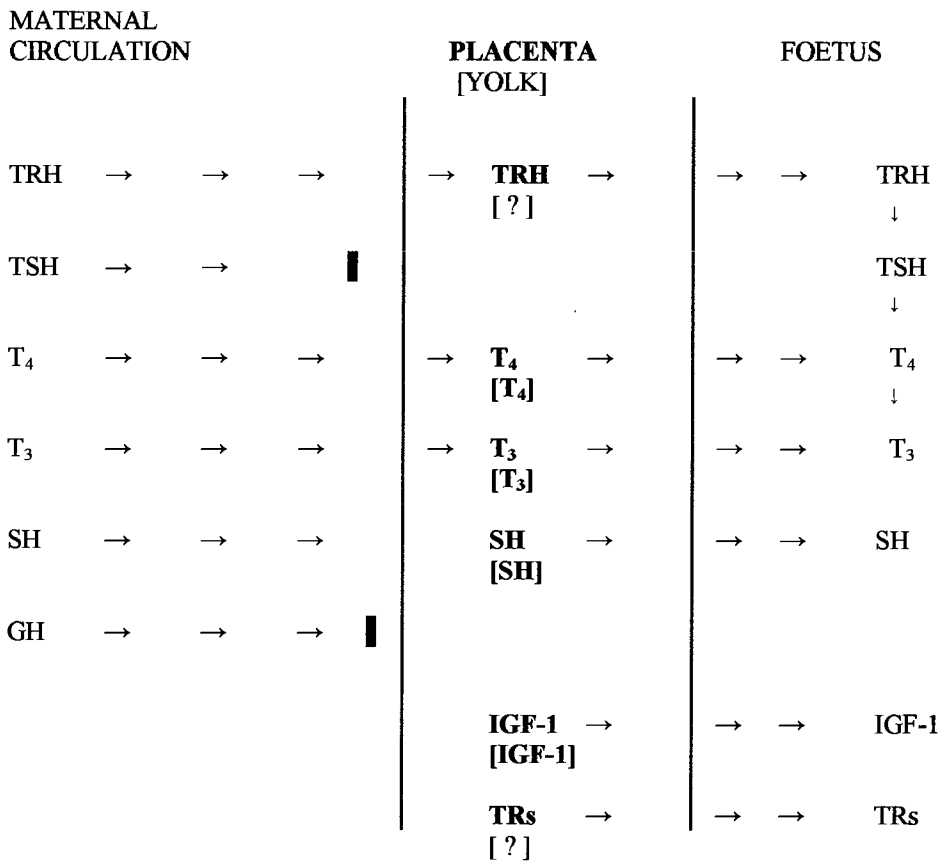

Figure 2.2. A summary of hormonal sources essential for embryonic development.

Substances known to pass through the placenta or manufactured by it indicated in **bold**, substances known to be stored in egg yolk in square brackets [ ]. TRH, *Thyrotopin-releasing hormone*; TSH, *Thyroid stimulating hormone*; T_3, *Triiodothyronine*; T_4, *Thyroxine*; GH, *Growth hormone*; TRs, *Thyroid hormone receptors*; SH, Steroid hormones (e.g. *oestrogen,progesterone*); IGF-I, *Insulin-like growth factor I*. Adapted from Chan and Kilby (2000, figure 2).

precursors), T_3 and T_4 all cross readily. T_3 stimulates the production of *epidermal growth factor* (EGF) as well as *oestradiol* (via affects on steroidogenesis) by the placenta. In fact, it seems that T_3 may play a key role in the development of the placenta itself (Kilby et al. 1998). In addition, all known TH receptors (TRs) are expressed by the placenta, which

manufactures a number of other substances critical to development, including steroid hormones (such as *progesterone, oestrogen, testosterone*) and *placental lactogen* (a growth factor related to GH; Hadley 2000).

The role of THs in somatic growth and embryonic development provides really significant insight into how species-specific developmental control can be achieved. Because of the critical role played by maternal THs in early embryonic development (both directly and indirectly), it is clear that the precise endocrine physiology possessed by a mother (or passed along into egg yolk) must control the early development of her offspring (Burrow 1997) and will continue to influence growth until they are born (Chan and Kilby 2000; Piosik et al. 1997; Wilson and McNabb 1997). Only from the point during development when the foetal thyroid gland becomes functional (which varies from species to species) do genes controlling TH function that were contributed by the sire have an opportunity to be expressed in their offspring.

The distinct stages at which offspring are affected by each set of parental genes controlling THRs (and thus growth and development) may provide a partial explanation as to why the phenotype of offspring from hybrid crosses differs depending on which species (or phenotype) is the dam, such as the documented differences between hybrid offspring from horses and donkeys (Asdell 1964; Epstein 1984), metamorphic and paedomorphic populations of an amphibian, the axolotl, *Ambystoma mexicanum* (Voss 1995), seasonally-and nonseasonally-adapted populations of white-footed mouse, *Peromyscus leucopus* (Wichman and Lynch 1991), and two species of mosquitofish (*Gambusia spp.*) that have distinct life histories (Scribner 1993).

Richardson (1995) recently reviewed the current status of knowledge on

developmental timing in different species of animals and found it to be surprisingly incomplete. Given the importance of heterochrony to vertebrate evolution, this state of affairs reiterates the importance of additional research in this area. Even Ernst Mayr seems to concur: he recently (1994) discussed a re-examination of the evolutionary concept of "recapitulation" in light of new discoveries in molecular genetics and developmental biology and came to the conclusion that increasing our understanding of how developmental timing works will be critical to evolutionary theory.

Life history strategies (including the specific developmental sequences that generate the organism as well as traits such as timing of birth and mating, preferred habitats, etc.) are known to have important evolutionary consequences, a point emphasized years ago by Gould (1977) in his seminal discussion of ontogeny and phylogeny. Although many researchers have pointed out that individual variation, life history strategies and evolution are inextricably tied (DeAngelis and Gross 1992; McNamara and Houston 1996), Gould (1996) has recently stressed that individual variation within species is *the* essential component that drives evolutionary change.

I have concluded, based on the evidence presented thus far, that pulsatile TH secretion is a prime candidate as the biological mechanism that coordinates species-specific growth and development. This mechanism might also explain the differential ability of some populations to change and adapt over time to changing conditions while others become extinct ("evolutionary evolvability"). It is apparent from the literature that we already know some such mechanism must exist. Hall (1992) comments that "...genetic variability, coupled with a developmental basis for polymorphism, and an ecological releaser, can provide plasticity for adaptive morphological novelty". De Pablo

(1993) pleaded years ago for more investigation on the function of hormones in development processes; she even presents a model to describe the role that a critical multi-functional hormone (such as TH) might play over the entire lifetime of an organism. She emphasizes that both the function and mode of action of such a hormone may "evolve" with the growing complexity of the organism as it ages, reminiscent of Parker and McKinney's (1999) discussion of individual ontogenies. I suggest that because of the multitude of physiological and developmental roles THs serve, and the changes in TH metabolism that occur over an individual's lifetime, THRs are a good candidate for the control mechanism we are seeking.

Ever since the role of steroid hormones in development and gene regulation was demonstrated (Evans 1988), the critical importance of hormonal interactions to evolution began to be more fully appreciated. For example, Finch and Rose's (1995) correlation of the "pleiotropic and epistatic effects of hormone and neural mechanisms regulating and scheduling life history pathways" (i.e. aging), recognizes many of the physiological inter-connections discussed here; however, his discussion tells us little about how these factors may have shaped populations as well as individuals. Real progress has been severely hampered by the lack of an appropriate theoretical framework that ties critical endocrine effects and interactions into an evolutionary context, a problem this work finally addresses.

2.6 Thyroid hormones and nutrition

One aspect of this model that merits further discussion is the relationship between TH production and nutrition. There are several ways in which nutrition can impact TH

physiology in all animals, including humans. The first is through consumption of foods that contain the essential amino acid *phenylalanine*; phenylalanine is converted to *tyrosine*, needed for both TH and melanin (pigment) production (Hadley 2000; Hemmer 1990). Consumption of foods containing significantly inadequate or excessive amounts of phenylalanine may be capable of acting as a selective force on a portion of a population that is sensitive to variable tyrosine levels (i.e. either increasing or decreasing the lifetime reproductive success of such individuals). Inadequate or excessive phenylalanine consumption over long periods of time may remove some individuals who are intolerant of such fluctuations from the pool of variants. Such a reduction in the total range of THRs represented might have slight developmental repercussions for the entire population, giving it unique morphological or behavioural characteristics (see also Section 3.1).

A similar situation might arise in relation to dietary sources of the iodine required for TH production, or through consumption of dietary flavinoids. Flavinoids are compounds found naturally in many foods, such as cassava (*Manihot esculanta*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), sweet potato (*Ipomoea batatas*), cabbage (*Brassica oleracea*) and other members of the genus *Brassica*, as well as fruit of the genus *Prunus* (especially apricots, cherries and almonds). Some flavinoids are converted by the gut into the goitrogen *thiocyanate,* a substance that interferes with normal TH function via competitive exclusion (thiocyanate has a similar three-dimensional molecular structure to thyroxine, Hadley 2000; see also Pedraz et al 1996). Occasionally, both effects may come into play, such as in Zaire, Africa. In many villages in Zaire, the extremely high incidence of goiter (enlarged thyroid glands associated with low TH

output (*hypothyroidism*), caused by insufficient dietary iodine), is exacerbated by a reliance on cassava as the primary source of calories (Geelhoed 1999).

Last, and perhaps most drastically, the thyroid function of individuals may be affected by direct consumption of THs from prey animals and their eggs (Eales 1997). THs are present in the flesh, blood, organ tissue, egg yolks and thyroid glands of all vertebrates and, unique among hormones, can be easily absorbed directly and without modification through the digestive tract when consumed by predators (Hadley 2000). The consumption of TH-rich foods must add considerably to an animal's daily TH load, especially for carnivores (but see Section 3.5 for a discussion of THs in kelp and other algae). Consumption of exogenous THs may explain the large disparity in turn-over rates for thyroxine between carnivores and humans mentioned previously (13-16.6 hours vs. 6.8 days). Carnivores obviously require an active metabolism capable of rapidly clearing the massive input of exogenous THs, perhaps coupled with a relative insensitivity to temporarily high levels or an ability to produce similarly high levels in the absence of exogenous hormone.

In all taxonomic groups, the changes in diet that commonly accompanied habitat shifts may have contributed to the precipitation of heterochronic speciation of all kinds and to the generation of developmental novelties characteristics of higher level taxa as well (new genera, families or orders). The wide availability of THs from external (environmental) sources is yet another factor that strongly implicates THs as the biological mechanism driving evolutionary change (see Section 3.5).

2.7 Genetic Control of Thyroid Function

A molecule as essential across all vertebrates as TH could not vary without disastrous repercussions and thus, it is not the TH molecule itself that varies within and between species but its pattern of production and utilization. Although it is clear that patterns of production must have a genetic basis, this is the area least well known of all aspects of TH metabolism.

As I have previously explained, iodine-bound TH precursors are manufactured and stored within the thyroid glands until *thyroid-stimulating hormone* (TSH) triggers the molecules to be hydrolyzed and released as T_4 and T_3. As a consequence, while the genes that govern the incorporation of iodine into THs operate within the thyroid glands themselves, the genes that control release of THs are located in the brain.

Ultimate control over TH secretion appears to come from complex interactions between the cells of the *suprachiasmatic nucleus* (SCN) of the anterior hypothalamus (located in the brain) and the neurohormone *melatonin* produced by the pineal gland (Korf 1994; Reppert and Weaver 2002; Wright 2002). Electrical stimulation of receptors in the retina relay signals about light to the pineal gland or the SCN; stimulation of receptors in the brain and peripheral nerves (CNS, *central nervous system*) do the same regarding temperature and emotional states (see Figure 2.1). The pineal gland responds to these combined signals with bursts of melatonin, which can be augmented or over-ridden by electrical and/or chemical output from the SCN (Haisenleder et al. 1992; Wright 2002): thus, neurohormonal stimulation from the SCN or the pineal stimulate pulsatile secretion of *thyrotropin-releasing hormone* (TRH) from the hypothalamus. Pulsatile release of TRH stimulates bursts of TSH from the pituitary, which stimulates pulsatile

release of THs from the thyroid glands.

Genetic control over this pulsatile hormonal cascade seems to come from eight or more so-called "clock genes," that reside in *circadian oscillator cells* of the mammalian SCN. The interaction of these genes with each other to generate a neurohormonal and/or electrical output appears to control basic *circadian timing* – the amazing mechanism that entrains so many biological functions to a 24-hour day. Because as-yet unknown "clock-modulating" genes are thought to exist as well (Reppert and Weaver 2002), the mechanism is far from being well understood. The SCN appears to be developed and functional in a number of laboratory animals by late foetal stage (e.g. rat, Shibata and Moore 1988; squirrel monkey, Reppert and Schwartz 1984) or at the very latest, by the early post-natal period (gray short-tailed possum, *Monodelphis domesica,* Rivkees et al. 1988). However, it cannot be emphasized too strongly that although circadian rhythms do modulate hormonal rhythms, *the two are not identical*; THRs are known to shift during development in amphibians even when light/dark cycles are constant (Wright 2002; also Gupta and Premabati 2002 for effects in a fish). Thus, it remains to be seen precisely which genes have dominant effects on THRs.

In addition, it is also apparent that the genetic basis for differences in overall TH metabolism does not lie exclusively in the genes that govern the secretion of THs. Variation is known or suspected to exist as well in the conformation or concentration of independent genes that control TH distribution in the blood and cerebral fluid (e.g. *transthyretin*), or that encode TH receptors, receptor ligands and/or cofactors in target tissues (e.g. Anderson et al. 2000; Huang et al. 2001; Wrutniak et al. 2001; Yaoita et al. 1990). There are many steps involved in getting TH molecules to target genes. Discerning

the variation that exists in the interdependent genes in the brain that govern secretion is much more difficult than identifying independent genes associated with TH transport (either through blood or cerebral fluid, e.g. Gagneux et al. 2001), genes of TH receptors within tissues or cells themselves (e.g. Flamant and Samarut 2003), or genes that require THs to regulate their function (e.g. Clark et al. 2003; Stedman et al. 2004; Uddin et al. 2004). All may be important to evolution, but I maintain that pulsatile TH secretion is the more significant (and thus deserves full investigation first) because of the ubiquitous role that TH availability, in a time- and dose-dependent manner, plays in developmental regulation across taxonomic groups.

CHAPTER 3. A NEW PARADIGM: THE THYROID RHYTHM THEORY

3.1. The thyroid rhythm theory

Individual variation for THRs within species is the key to proposing a scenario in which animals could be exposed to the selection pressures necessary for the biological changes associated with both protodomestication and heterochronic speciation to occur. I suggest that slight variations in THRs among individuals are reflected in phenotypic differences of a physiological nature, i.e. manifested as differences in the response of individuals to stress and stimuli provided by the external environment, as well as to internal physiological stresses (such as needs for increased THs required for pigment production, foetal growth, neonatal growth or gonad maturation). Such THR polymorphisms (probably controlled by several genes) are heritable traits that manifests themselves also as variation within species for traits such as size, coat colour, and the coordinated timing of ovulation and moult.

I contend that the existence of variability within species-specific THR phenotypes provides a mechanism for rapid adaptation of populations to environmental change or to totally new environments – that the stresses and stimuli associated with colonization of radically new territories in particular (utilizing new food sources, adjusting to new predators and competitors etc.) provide the selection pressures that divide a population according to specific physiological phenotypes. During expansion into a new habitat, I propose that the colonizing subset of any population will be composed only of those individuals who possess the physiological ability to tolerate increased stress and stimulus loads, while the group remaining in the old habitat retains a full complement of stress-tolerant and intolerant phenotypes (just because *some* stress-tolerant individuals leave to

colonize a new territory, doesn't mean that *all* stress-tolerant individuals within an ancestral population actually leave). Individuals that can tolerate the stresses of a new habitat (including anthropogenic environments) constitute a similar non-random segment of any population. When interbreeding among these physiologically-similar animals occurs within a small founder population, offspring can inherit only a limited complement of alleles for the genes controlling THRs. Without a full range of THR variability, descendants of this founding population are almost certain to be behaviourally, reproductively and morphologically different from the ancestral population after relatively few generations, with the well-coordinated suite of differences between them recognizable as classic heterochronic changes of one kind or another.

3.2. Domestication as a speciation process

The model depicting domestication as a biological process (Figure 3.1, simplified in Appendix A, Figure 4) defines both incipient species and incipient domesticates (stage 1) as equivalent population subsets of wild source populations (stage 0) that colonize new habitats. However, there are differences in the nature of the new environments they invade. For incipient domesticates, these environments are *anthropogenic*, a term that describes a localized set of environmental conditions created by the physical effects of permanent or semi-permanent human settlement (Horwitz and Tchernov 1991; Tchernov 1993a,b). An anthropogenic environment is also a habitat dominated by the continuous presence or proximity of people. In contrast, for incipient species the new environment may be either a previously unoccupied adjacent niche or a niche newly created by environmental or climatic change.

In both cases, the new habitat offers resources unavailable or scarce in the original or source territory (such as food and breeding sites), making it highly attractive. In both cases, individuals within the source population who have the highest physiological tolerance to stress (i.e. possess a *particular* THR phenotype) are those most likely to invade a new territory (see also Crockford 2002b, Appendix A; figure 3).

The division of the source population during colonization of a new habitat (via classic allopatric and peripatric speciation) is thus distinctly *non-random*. Non-random subdivision of the source population according to existing variation in THR phenotypes (and the genes that control them) makes this model distinct from speciation described by geneticists who, along with Ernst Mayr and others, helped shape the so-called "modern evolutionary synthesis": John Haldane, Ronald Fisher, and Sewell Wright (summarized in Schwartz 1999). These geneticists, because they used mathematical models to predict the effects of selection over time based on the frequency of alleles in a population, assumed that mating was random among individuals (this is stated explicitly). They also appear to have assumed a process of random subdivision of populations during a split, which I conclude partly because they do not state explicitly otherwise and because they do not state a basis for suggesting that certain alleles would necessarily group together. This distinction between my model and theirs (non-random vs. random population subdivision) is significant and stems largely from the fact that my model is *not* mathematical.

In my model, interbreeding within a small, isolated population of only stress-tolerant individuals rapidly establishes a new mean pattern and range of variation for THRs in the colonizing group. This shift precipitates significant changes in descendants

because of the intimate connection that exists between the adrenal stress-response system, development and THs. Descendants of founders are recognizable as different from their ancestors within relatively few generations because shifts in THR phenotypes within small populations have morphological, behavioural and reproductive repercussions. Viewed from this perspective, it is apparent that in both heterochronic speciation and protodomestication, the initial populations of ancestors are equivalent subsets of wild source populations (stage 1) and the selection mechanism is the same. Thus, descendants of both processes are equivalent entities that must qualify as equally *real* species (see species definitions, Section 1.4).

It is also apparent that stage 2 - the point at which gradual adaptation to the new habitat occurs - is the first point of departure between the two processes, although the magnitude of difference is small. While some might still argue that deliberate human selection played a significant role in the changes that affected incipient domesticates during stage 2, the prevailing view now concedes that natural selection was probably more significant (Clutton-Brock 1992a). If human selection did occur, it was probably *unconscious* (Darwin 1868; Zohary et al. 1998). Unconscious selection refers to, among other examples, choosing to slaughter individuals with intractable temperaments (thus removing them from the breeding pool), or sparing from slaughter individuals that are good mothers or produce large litters.

In other words, adaptation of incipient domesticates to the conditions within their anthropogenic environment proceeded primarily via the same mechanisms of natural selection that adapt incipient wild species to their new environments. However, anthropogenic environments do possess some inherent properties that may not be present

	SPECIATION	PROTODOMESTICATION
Stage 0	WILD SOURCE POPULATION [a large group of individuals characterized by a unique mean TR and a wide range of variation in TR phenotypes], which contains within it a number of potential...	WILD SOURCE POPULATION [a large group of individuals characterized by a unique mean TR and a wide range of variation in TR phenotypes], which contains within it a number of potential....
	FOUNDER INDIVIDUALS (ANCESTORS) [a group of individuals who share a stress-tolerant TR phenotype, i.e. a non-random subset of the wild source population]	**FOUNDER INDIVIDUALS (ANCESTORS)** [a group of individuals who share a stress-tolerant TR phenotype, i.e. a non-random subset of the wild source population]
Stage 1	These founders colonize a new environment & the lack of variation in TR phenotypes within this split-off population rapidly establishes a new mean TR phenotype for the group & as a consequence, change via...	These founders colonize an anthropogenic environment & the lack of variation in TR phenotypes within this split-off population rapidly establishes a new mean TR phenotype for the group & as a consequence, change via...
	HETEROCHRONIC PROCESSES into a unique descendant form, the...	HETEROCHRONIC PROCESSES into a unique descendant form, the...
	INCIPIENT WILD SPECIES	**INCIPIENT DOMESTIC SPECIES**
Stage 2	this population expands into its new *territory* & *adapts through*...	this population adapts to its anthropogenic *habitat* through a combination of...
	NATURAL SELECTION	NATURAL SELECTION & UNCONSCIOUS HUMAN SELECTION
	establishing a unique...	establishing a unique...
	FOUNDATION WILD SPECIES [a group with a mean TR phenotype & range of TR variation distinct from its ancestor, with intrinsic reproductive isolating mechanisms]	**FOUNDATION DOMESTIC SPECIES** [a group with a mean TR phenotype & range of TR variation distinct from its ancestor, with intrinsic reproductive isolating mechanisms]
Stage 3	geographic expansion due to population growth eventually leads to the formation of...	geographic expansion via migration with humans eventually leads to the establishment of....
	GEOGRAPHIC SUBSPECIES [each group containing most of the TR phenotypes of the foundation species]	**REGIONAL VARIETIES** [each group containing most of the TR phenotypes of the foundation domesticate]
Stage 4	Further population growth, environmental change or competitive pressures may lead to additional colonization events, generating additional INCIPIENT SPECIES via...	People take control of environmental conditions, population structure & breeding, consciously splitting off subsets of similar physiological phenotypes or INCIPIENT BREEDS, via
	HETEROCHRONIC PROCESSES & NATURAL SELECTION	DELIBERATE HUMAN SELECTION [via isolation of specific heterochronic traits & fixation of rare mutant alleles, etc.]
	leading to the development of further	leading to the development of numerous
	POLYMORPHIC SPECIES [each with distinct TR phenotypes kept distinct via adaptation to unique environmental conditions]	**POLYMORPHIC BREEDS** [each with slightly different TR phenotypes kept distinct only because of human-mediated artificial isolation]

Figure 3.1. My model for the role of thyroid rhythm (THR) phenotype selection in heterochronic speciation and protodomestication.

in the wild, including the constant physiological stress of close association with humans, the constant immunological stress of very close association with conspecifics, the inbreeding stress imposed by limited choices of mates, and the nutritional stress imposed by severely limited food resources, especially for confined populations. Therefore, the selective forces operating on incipient domesticates will be different from those acting on incipient wild species, even if the actual process of adaptation is the same.

Ultimately, geographic radiation of established wild species will occur as their population size increases; the subsequent adaptation of geographically distant subpopulations to their local environments leads to the development of distinct *subspecies* (stage 3). For established domestic species, whose geographic radiation is limited by associated human radiation and migration, population increases and adaptation must take place within the limitations of each anthropogenic environment, leading to the development of what are usually called *local regional varieties*.

It is not until stage 4 that the greatest difference in the history of the two types of descendant species occurs. This is the point at which humans assume absolute control over domestic species and, through deliberate artificial selection, develop distinctly polymorphic breeds. As I discuss in more detail elsewhere in relation to dogs (Section 3.3 and Crockford 2000a), humans have been able to discern and then artificially isolate subsets of individuals with similar physiological phenotypes (incipient breeds) that share behavioural, morphological and reproductive traits (Coppinger and Schneider 1995; Coppinger and Coppinger 2001). Artificial isolation of specific THR phenotypes - whether people realize this is what they are doing or not - allows humans to initiate and perpetuate heterochronic processes decoupled from nature. This kind of selection, along

with fixation of rare mutant alleles through inbreeding and hybridization of existing breeds, has resulted in a truly astonishing number of domestic variants in some animals, including more than four hundred recognized breeds of dogs and almost as many pig breeds (Alderson 1978; Wilcox and Walkowicz 1989; Wayne 1986c).

However, the polymorphic breeds resulting from human manipulation stay distinct only if artificial isolation is maintained (stage 4). In contrast, the polymorphic wild forms of stage 4 are true species that stay distinct because they are uniquely adapted to specific habitats and have reproductive cohesion mechanisms (as discussed in Section 1.1).

As the model summarized in Figure 3.1 emphasizes, the heterochronic changes associated with protodomestication are significant consequences of the evolutionary process involved. Animals that do not manifest the distinctive biological changes associated with protodomestication, such as Asian elephants (Olivier 1984; Reed 1984), the bottle-nosed dolphin, *Tursiops truncatus* (St. Aubin et al. 1996), and reindeer (Skjenneberg 1984), have not undergone this process as described and are therefore more appropriately referred to as *managed species,* not true domesticates.

I have changed my position somewhat on a statement made elsewhere (Crockford 2002a) that managed species also include those domesticates for which historical records indicate humans deliberately removed randomly selected animals from the wild and subsequently maintained populations in captivity, such as for the Syrian or golden hamster (Clutton-Brock 1992b). Details on founding conditions for the domesticated golden hamster (Robinson 1984b) suggest that initial mortalities suffered in the first generation (an adult female and eight young reduced to one surviving male and two females, in 1930) could actually have been a "selected mortality" event, analogous in

some essential ways to the "self-selection" for stress-tolerance experienced during protodomestication in other taxa. In selected mortality cases, rather than stress-tolerant colonizers leaving intolerant cohorts behind, the stress intolerant portion of the population leaves via death, so that stress-tolerant colonizers alone remain in the new habitat. There is still non-random subdivision of the source population according to physiological phenotype that leaves only stress-tolerant individuals as founders, but the factor of choice present in the colonization case does not exist for populations undergoing selective mortality.

The fact that domesticated hamsters do have somewhat ameliorated behaviour and many coat colour variants compared to wild conspecifics would tend to support this interpretation (Künzi and Sachser 1999); descendant populations have indeed changed in some physical and behavioural respects as a result of being raised in captivity and so probably qualify as valid examples of protodomestication. A similar situation of high initial mortality in a captured population, described below in Example 3, may explain the rapid changes in behaviour and physiology seen in farmed salmonids.

In some domesticates, however, significant biological differences from wild forms cannot be substantiated. Obviously, when the ancestral animal has not been clearly established, as for the horse (Bőkőnyi 1984), it is not possible to compare biological features. These animals require further investigation to establish their taxonomic status (as discussed in Gentry et al. 2004 in regards to the naming of wild animal species and their domestic derivatives). In other taxa, however, morphological, physiological and behavioural differences between ancestors and descendants are present but just not so definitive, as occurs for example in all camelid species, the turkey, and in the cat

(Crawford 1984; Fowler 1989; Heath 1989; Mason 1984c; Robinson 1984a; Smith 1989; Vidal-Rioja et al. 1994). These taxa are simply less convincing examples of the protodomestication process under discussion.

However, the biological differences between wild taxa and the natural domestic descendants under consideration here can be clearly established for dogs (Clutton-Brock 1995; Morey 1992, 1994), pigs (Epstein and Bichard 1984; Jensen 2002), cattle (Epstein and Mason 1984; Loftus et al. 1994), sheep (Ryder 1984), goats (Mason 1984b; Melinda 2000), and the lesser known Asian bovids - water buffalo and mithan (Olsen 1993; Ross Cockrill 1984; Simoons 1984). These examples are adequate for illustrating the range of ecological and physical types that have undergone similar biological change as a result of protodomestication (e.g. carnivorous vs. herbivorous/omnivorous feeders, forest vs. savannah/steppe dwellers).

The morphological changes to animals produced through protodomestication include significant modification of the skeleton. Consequently, archaeological remains are an essential source of evidence for unravelling the history of domestic species (Crabtree 1993; Davis 1987). However, the archaeological record of skeletal remains does not show the clear intermediate stages between wild and domestic forms we are led to expect from the cultural explanation of domestication, which assumes that changes are gradual (Clutton-Brock 1992b; Morey 1992; Tchernov and Horwitz 1991). Admittedly, there is controversy over some data (e.g. Dayan 1994). However, the absence of clear intermediate forms in *all* taxa (despite large numbers of samples for some) suggests that the physical changes associated with protodomestication were not gradual but abrupt, implying that the process must have been relatively rapid.

Archaeological deposits are usually dated by the analysis of a specific isotope of carbon remaining in various organic materials found within them. A ^{14}C date with a standard deviation of 100 years (e.g. 11,000 ± 100 years) describes the 200 year range within which the date is estimated to fall (Fagan 1994; Wintle 1996). Chronological dates for the earliest archaeological material of major mammalian domesticates (e.g. dogs, pigs, cattle, sheep, etc.) are associated with standard deviations that vary from less than 100 to slightly more than 800 years (Clutton-Brock 1995; Davis and Valla 1978; Reed 1984). These statistical error ranges given for all dates of archaeological specimens are problematic (Crabtree 1993) because it means we cannot determine if specimens with close radiocarbon dates are actually chronologically contemporaneous or sequential. It does, however, mean that the process of speciation associated with protodomestication could have been accomplished in 200 years or less, but probably took no more than 1,600 years.

There are non-domestic populations in which similarly rapid speciation rates appear to have occurred. For example, the distinct forms that make up "species flocks" of fish from the family Ciclidae in some African freshwater lakes are now estimated to have developed in as little as two hundred years (Owen et al. 1990) – a figure very much less than an earlier estimate of 2,800 years per speciation event for these fish, a figure Mayr (1988:392) considered the "most rapid rate imaginable." The "species complex" comprised of distinct marine and freshwater forms of three-spine sticklebacks may eventually prove to show similarly rapid rates (McKinnon and Rundle 2002). Thus protodomestication and known cases of especially rapid speciation appear to operate within similar time scales.

In contrast to morphological, ecological and behavioural differences, the genetic distinctions between wild and domestic taxa are much less clear. Modern domesticates are generally very similar genetically to extant species of their wild ancestors. It must be emphasized, however, that extant species of some taxa (such as modern wolves) are not the ancestral species of the domestic form (Morell 1997), but simply their closest living relatives (just as the chimpanzee is our closest living relative, not a direct ancestor). Similarly, modern domestic breeds are not exact genetic equivalents of their primitive domestic ancestors, especially in those taxa under consideration here that have been subjected to artificial selection, cross breeding and human-mediated migration for thousands of years.

Nevertheless, there have now been comparative studies examining genetic diversity and ancestral relationships for the dog, cattle, water buffalo, sheep, horse, goats, and pigs, as well as the South American camelids (Bradley et al. 1998; Giuffra et al. 2000; Hiendleder et al. 2002; Jansen et al. 2002; Kadwell et al. 2001; Koop et al. 2000; Luikart et al. 2001; MacHugh and Bradley 2001). Comprehensive analyses have been undertaken so far for dogs, cattle and horses, and these studies have utilized tissue samples from extinct as well as extant individuals. None of these studies (except the camelid investigation, which did not address the issue) supply support for single domestication events followed by extensive human-mediated dispersal. For example, Loftus et al. (1994) found that the traditional explanation for the domestication of cattle (as a single domestication event followed by dispersal, with humped varieties derived later as local adaptations to arid conditions, Epstein and Mason 1984) was not supported by their genetic studies. They found mitochondrial DNA (mtDNA) evidence for two

distinct domestication events, one in Europe that produced cattle varieties without humps (presumably from the aurochs subspecies *Bos primigenius primigenius*) and another in India that produced humped cattle varieties (from *B. p. namadicus*). The study produced no clustering of mtDNA haplotypes according to breed in either type of cattle.

This evidence supports the taxonomic distinction that has been made between zebu-type (humped) cattle as *Bos indicus* vs. taurine-type (those without humps) cattle as *Bos taurus*, except for African zebu-type breeds. African zebu-type breeds were found to have taurine-type mtDNA haplotypes, a pattern most easily explained by proposing European origins for African cattle populations that were subsequently modified by introgression of Asian zebu genes through male transmission. Male transmission would have passed along the zebu morphotype while the taurine mitochondrial genotype was retained, due to the maternal inheritance of mtDNA. The large sequence divergence between the two *Bos* lineages was interpreted by the authors as evidence for at least two domestication events for cattle, from two subspecies of aurochs which had been geographically and genetically distinct for thousands of years prior to the domestication events. Subsequent work (Bradley et al. 1998; MacHugh and Bradley 2001), which included sequences from extinct individuals as well as Y-chromosomes, strengthens even further the case for a minimum of two events involving genetically distinct subspecies of aurochs in Europe and western Asia (similar results for horse origins, Jansen et al. 2002).

Koop et al. (2000) similarly present mtDNA evidence for at least two, and perhaps as many as five domestication events for the dog, with some evidence of introgression between genotypes. Significantly, there were no mtDNA haplotypes clustering according to breed in this study, refuting predictions of polyphyletic origins for the dog based on

breed morphotypes (Clutton-Brock 1981; Morey 1994). This was the first study on dogs to use prehistoric samples as well as modern ones, although we now have additional data of this type (Leonard et al. 2002). Koop et al. comment that the presence of wolf sequences in several "dog" sequence clades generated by previous researchers dating the divergence of dogs from wolves (Vilà et al. 1997) precludes the use of "molecular clock" estimates – that evidence in the study of hybridization between dogs and wolves violates one of the basic assumptions required for use of that technique. I discuss other problems associated with unravelling dog/wolf genetic relationships elsewhere (Crockford 2000b).

The studies described above, which present evidence that the genetic history and presumed historical relationships of most domestic animals do not correspond, is not a situation unique to domesticates. Studies of several wild species that were expected to show genetic support for their ecologically divergent histories (East African chimpanzees (*Pan troglodytes*), Goldberg and Ruvolo 1997; southern hemisphere fur seals (Otariidae), Lento et al 1997; Lake Tanganyika ciclid fishes (Ciclidae), Kirkpatrick 2000; Rűber and Adams 2001) or their ecological and historical similarities (east African black-backed jackals (*Canis mesomelas*), Wayne et al. 1990; Pacific harbour seal (*Phoca vitulina*), Lamont et al. 1996) did not show the anticipated mtDNA patterns. Possible explanations offered for the lack of genetic congruity include unexpectedly high gene flow (including hybridization) between populations, incomplete lineage sorting, longer than expected population separation and variable rates of mtDNA mutation. Heyning (1997) has recently suggested (for whale phylogenies) that inappropriately rooted gene trees may also generate conflicting results. The possibility that multiple speciation events could also have produced the genetic results generated in these studies is simply not considered,

except perhaps by Rűber and Adams regarding their study on Lake Tanganyika ciclid fishes.

In general, however, as I discuss fully elsewhere (Crockford 2000b), because natural domesticates and their extant ancestral species are so close genetically, hybridization between them almost always produces viable offspring (e.g. for sheep and goats, Hemmer 1990; wolves and dogs, Iljin 1941; llamas and alpacas, Kadwell et al. 2001). However, the ability to hybridize with systematically close relatives is not by any means restricted to domesticates and should not (by itself) indicate that domesticates are not valid species (see discussion in Section 1.1). In fact, one of the unexpected results of recent molecular genetic studies has been the ability to identify previously unrecognizable hybrids between wild species. Natural hybridization has now been documented between wolves and coyotes, *Canis lupus vs. C. latrans,* (Lehman et al. 1990; Wayne and Jenks 1991; Wayne and Gittleman 1995), blue and fin whales, *Balaenoptera musculus vs. B. physalus* (Arnason et al. 1993), red-backed and bank voles, *Cleithrionomys rutilus vs. C. glareolus* (Tegelstrom 1987), and Arctic char and brook trout, *Salvelinus alpinus vs. S. fontinalis* (Bernatchez et al. 1995), among others. Hybridization is much more common among otherwise valid species than previously realized (e.g. Arnold 1997; Levin 2002).

If apparent genetic differences are so small, however, what accounts for the significant biological differences between wild and natural domestic taxa? As previously stated, the answer appears to be that significant shifts in developmental growth rates or timing of developmental events have occurred. The biological evidence thus suggests strongly that the essence of protodomestication is heterochronic speciation, while the archaeological evidence suggests this speciation was implemented exceedingly rapidly. I

outline several examples in more detail below, which I hope will clarify some pertinent details of the concept.

3.21 Origin of the dog

As dogs were the first and most widely distributed natural domesticate, the associated archaeological and paleontological record for them is the longest and most complete. Also, a modern representative of the ancestral species exists and there is thus a wealth of experimental behavioural, physiological and genetic data available for both species (Crockford 2000a).

We now have solid evidence from a number of studies (including genetics and behaviour) that the wolf (*Canis lupus*) is the direct ancestral species of all forms of dog (*C. familiaris*) (see Gentry et al. 2004 for a discussion of this taxonomy). Distinctive reproductive physiology and behaviour provide intrinsic cohesion mechanisms within both species (Asdell 1964; Arons and Shoemaker 1992; Boitani et al. 1995: Fox 1978; Gittleman 1989; Steinhart 1996), despite the very close genetic relationship between them. Both wolves and dogs prefer to mate with members of their own species; when hybridization does occur, it is almost always unidirectional from wolf males to dog females (Crockford 2000b; Hemmer 1990; Iljin 1941; Mengel 1971; Wayne and Jenks 1991), although the reverse occasionally occurs.

It has now been shown that more than one speciation event was probably involved in the origin of the dog, and perhaps as many as five (Clutton-Brock 1992b, 1995; Koop et al. 2000; Leonard et al. 2002; Morell 1997; Pennisi 2002). The earliest undisputed evidence for the dog as a distinct taxon is a mandible from a German grave site dated ca.

14,000 B.P. (Benecke 1987; Clutton-Brock 1995), supported now by a few additional specimens of this age from the Czech Republic (Musil 2000) and Siberia (Sabline and Khlopachev 2002). Several specimens from the early Natufian period are considered the oldest dog specimens from Israel, all of which date to ca. 11,000-12,000 B.P. (Tchernov and Valla 1997).

During the period 10,000-7,000 B.P., there are a large number of undisputed dog finds from many places around the world, including: Iraq, at 9,250-7,750 B.P.; China, at ca. 7,000 B.P.; Chile, at 8,500-6,500 B.P.; England, at 9,940- 9,490 B.P.; Germany and Denmark, at ca. 9,000 B.P.; Japan, at ca. 8,000 B.P. and the continental United States, at ca. 10,000-8,000 B.P. (Clutton-Brock 1995; Morey and Wiant 1992). These early primitive dogs show some size variation between regions, but in general appear to be similarly dingo-like, unspecialized animals (Clutton-Brock 1995); northern European and Chinese specimens tend to be the largest (dingo-sized), Japanese (and some North American) specimens the smallest (terrier-sized), while western Asian samples are somewhere in-between (Crockford 1997, 2000b). Distinct morphotypes of dogs (such as giant mastiffs, gracile sight hounds and toy-sized lap dogs) are not apparent until much later, in general not emerging until between 3,000-4,000 years ago in most areas (Crockford 2000b; Clutton-Brock 1995).

Dogs have been shown to be paedomorphic in relation to wolves, based on several studies of behaviour, growth and skeletal anatomy (e.g. Coppinger and Feinstein 1991; Coppinger and Schneider 1995; Morey 1990, 1992, 1994). This means that the most obvious differences between the wolf and dog are the result of the dog maturing at a stage of development equivalent to that of a juvenile wolf, in both morphology and behaviour.

Robert K. Wayne showed, in a pivotal study, that these differences appear to have been caused by an inherited reduction in growth rate implemented primarily during late foetal development and the early postnatal growth period (Wayne 1986a, b, c).

Wayne undertook a series of comparative studies using several wild canid species and a few dog breeds and showed, for example, that small paedomorphic breeds (such as the Lhasa Apso) are smaller because they are not only born somewhat smaller than average but grow very slowly immediately after birth. In addition, they mature physiologically and skeletally before one year of age. In contrast, giant breeds (such as the Great Dane) are born slightly larger than average but in addition, grow exceedingly rapidly after birth. The growth of Great Danes slows down after the early postnatal period (to a rate that is similar in all breeds) but continues well into the second year of life, so that they are not fully mature until about two years of age. Other breeds often fall in between these two extremes of modified growth, as the first primitive dogs probably did. The gestation length for all dog breeds (large and small) is the same (60-63 days), suggesting that inherited differences in growth rates account for size and shape distinctions between breed morphotypes. In contrast, small wild canids such as foxes (*Vulpes/Alopex spp.)*, which have postnatal growth rates similar to much larger wild canids, appear to be smaller because their gestation period is shorter by about 10 days (a similar pattern of shorter gestation in small representatives of a given lineage has also been noted by Kurtén (1988).

Goodwin et al. (1997) have recently provided some experimental evidence that dog breeds with extremely paedomorphic wolf morphology also possess more distinctly juvenile wolf behaviour, with other breeds falling in between the two extremes. An

explanation for such variation in breed behaviour is offered by Coppinger and Schneider (1995), who emphasize that juvenile wolves are animals in transition: they are undergoing a metamorphosis of sorts from neonate (with one set of unique behaviour and motor patterns) to adult (with a new set of behaviour and motor patterns). Neonatal patterns disappear and are replaced by adult patterns during this transition period, so that juveniles retain some motor patterns and behavioural attributes of the neonate while they add adult attributes (e.g. juveniles at a certain stage can both suck and chew). Juveniles are thus endowed with an especially wide range of motor skills and behaviour.

Thus, inherited controlled growth patterns of breeds which leave an adult dog maturing at an early juvenile stage would produce a different repertoire of behaviour and motor patterns than one which matures at a later juvenile stage. Coppinger and Schneider use this model to explain the differences in behaviour and motor patterns evident in the plethora of breeds that have emerged within the past 100 years, and in cross-breed (i.e. hybrid) offspring of different dog breeds. They suggest that hybridization between breeds that possess unique inherited programmed growth (associated with both morphological and behavioural consequences), can create truly new patterns of inherited behaviour in the offspring, which in breed-creation programs can be set by subsequent inbreeding and selection.

Part of Wayne's study (1986c) emphasized that the morphological variation in skull shape evident between the adults of dog breeds representing distinct morphotypes (such as the extremely paedomorphic Lhasa Apso and the German Shepherd Dog) is a reflection of the variation in skull shape of the wolf as it matures from neonate to adult. In other words, the dramatic shape change during ontogeny that occurs in the wolf is what

has allowed artificial selection to fix so many skull shapes in modern dog breeds. A similar situation also exists for the pig, where the dramatic shape change in the skull during ancestral ontogeny (from rounded to elongate) is reflected in a remarkable number of modern pig morphotypes.

In contrast, animals such as the horse and cat (where there is only a small amount of skull shape difference between neonate and adult) would not be expected to show as much skull shape variation between modern breeds. As Wayne emphasizes, neither horses nor cats exhibit anywhere near the amount of morphological variation as dogs and pigs. Archaeological material of primitive domestic horses and cats, therefore, might be expected to differ less in size and shape relative to their wild ancestors than other animals (given that the same heterochronic process is at work), making the recognition of early domestic forms especially difficult in these taxa, and indeed this seems to be the case (e.g. Clutton-Brock 1981).

In conclusion, research on dogs and wolves suggest that heterochronic processes account for all of the morphological, physiological, and behavioural differences between them, and also for the differences between dog breeds subsequently developed through artificial selection. In addition, the same patterns evident in dog evolution are also seen in the other domestic mammals included in this discussion. Thus natural domesticates constitute some of the best-known and important examples of heterochrony and as a consequence, understanding how and why protodomestication occurs may shed significant insight into heterochrony as an evolutionary process.

In the case of protodomestication, however, these heterochronic changes have a specific directionality. Incipient domesticates are all distinctly paedomorphic because the

anthropogenic stress component is the same in all cases, for all taxa. Human settlements created unique environments in evolutionary terms, ones which had never been available to wild animals before, and the proximity of humans constantly operating within these new habitats was also a new kind of environmental stress. The selection pressure imposed by the stress of constant human proximity on founders would have become increasingly more intense as time passed, driving out individuals with only marginal tolerance, because as the population of both people and incipient domesticates (such as wolves) increased in numbers, the frequency of encounters between them would also have increased.

The reproductive isolation of the colonizing group does not have to be total for this process to work, but introgression of the genes of a few stress intolerant individuals would undoubtedly slow the process down, especially during the early stages. With much introgression (indicating relaxation of the resource competition or other factors that precipitated the initial invasion), the process could essentially reverse itself. Thus the conditions required to precipitate protodomestication (through association of wolves or any other animal with human settlements) could have occurred many times without initiating permanent changes.

While this hypothesis doesn't preclude the possibility that protodomestication of any single taxon could have happened more than once, is doubtful that it happened often. The environmental and/or population pressures on the animals would have to have been quite severe and the attractions of the human-dominated habitat very strong to encourage wild taxa to expand into an anthropogenic environment. In addition, the stress-tolerant (i.e. less fearful) behavioural types must have been present as natural variations in the

ancestral population to start with (populations of animals who all tolerate this kind of stress well - or very poorly - do not lend themselves to protodomestication-type speciation). This combination of necessary factors could explain why colonization of anthropogenic environments by wild taxa happened rarely overall in relation to the number of potential animal species available for domestication (cf. Diamond 1999, 2002).

3.22 Origin of sheep, goats and cattle

I suggest that protodomestication of sheep, goats and cattle proceeded in a manner similar to that proposed for the speciation of mithan from gaur (a type of wild Asian cattle). The method of handling modern domestic mithan stocks in Bangladesh and Burma (described by Simoons 1984) appears to perpetuate the initial protodomestication process. Mithan, like their wild gaur ancestors, are attracted to salt. People today manage their mithan stocks in the most minimal fashion: they simply encourage the animals to return to the centre of habitation areas by providing salt. No attempts are made to handle or tame mithan, or to confine them - because the regular proximity of the animals adequately facilitates occasional culling of individuals for ritual use (they are not eaten regularly). The animals feed on available wild forage in the forest and breed entirely at will and yet, mithan are classic domesticates: they are smaller than wild gaur, relatively docile in behaviour and often piebald.

While it is possible that previously domesticated mithan stocks are merely maintained by this system, there are compelling reasons for protodomestication of wild guar to have occurred under these (or similar) conditions. This method provides both of the components necessary for precipitating protodomestication changes as described here:

an attractive resource not readily available in the wild (salt) and the constant proximity of humans to provide the selection pressure. For other bovid taxa such as cattle, goats and sheep, the attractant could have been either the newly domesticated grain crops under cultivation during the Neolithic (Reed 1984; Tchernov 1993a, b), deliberately supplied salt, or the salt in human urine that would have accumulated naturally around settlements (as Zeuner (1963) has noted, human urine is known to strongly attract reindeer and seems to encourage herds to associate closely with nomadic Sami tribes). Cultivated crops are especially likely as an attractant if stands of wild grain in the area had been depleted by previous centuries of human collection, as appears to be the case (Harris 1996); if so, accumulations of human urine may have been an added bonus.

3.23 Farmed and hatchery salmonids

Modern "domesticated" rainbow trout (*Oncorhynchus mykiss*) descend from wild eggs of coastal steelhead collected from a number of sources in US western states in the late 1800's (Behnke 1992), a founding population that probably experienced an initial high mortality (perhaps as high, or higher than, 80%) in response to the crowded conditions experienced during initial captivity (Gary Thorgaard, pers. comm., Nov. 1999). Thus, all domestic trout are descendants of the few animals physiologically capable of surviving the stressful conditions of initial captivity; such selective mortality (also seen in the deliberate domestication of the golden hamster, as discussed earlier) may be analogous to population subdivision according to physiological tolerance to stress described for classic protodomestication (although in this case, stress-intolerant animals simply die).

Marked differences in behaviour exist between modern domestic trout and wild rainbow (Behnke 1992), a phenomenon seen in other captive-reared salmonids (Gross 1998; Moyle 1969; Vincent 1960). For example, a tank in which twenty or more domestic trout thrive can hold only two wild-caught fish for any length of time (Thorgaard, pers. comm.., and personal observation, Washington State University Zoology Department trout research facility, 1999), suggesting that most wild fish need considerable inter-individual space (often more than is economical for captive populations). In addition, while wild-caught fish retreated to the bottom of the holding tank when the lid was raised and attempted to hide in the shadows, domestic trout were so vigorous in their rush to the surface that some leaped clear of the water. This behavioural difference suggests that most wild trout have a natural fear of activity at the surface of the water that compels them to dive and seek shelter, a predator-avoidance response that may be associated with or linked to the stress response to crowding. Thus, mortality of individuals in the original captive population that were sensitive to the stress of crowding may have simultaneously eliminated those individuals with a high predator avoidance response; the facilities used for raising wild-caught trout eggs in captivity may have unintentionally selected for those few animals that lacked extreme stress responses of all kinds. As a consequence, the descendants of this population are now amenable to being fed and otherwise tended from the surface of holding tanks without stress and can be held in concentrations high enough to make trout farming economically viable (e.g. Gross 1998).

Recent study has also shown that farmed rainbow trout have markedly different brain morphology compared to wild fish, suggesting that domestication has affected brain development after little more than 100 years (Marchetti and Nevitt 2003). There is

evidence, however, that even seventh generation farmed Atlantic salmon (*Salmo salar*) show faster growth rates and increased levels of growth hormone compared to wild fish (Fleming et al. 2002; McGinnity et al. 2003), changes that have appeared after less than 25 years in captivity. Even hatchery-raised Chinook salmon (*Oncorhynchus tshawytscha*) have been shown, after only a few generations, to lay more eggs that are smaller in size than wild salmon (Heath et al. 2003; Fleming et al. 2003). Smaller eggs are more vulnerable to predation than larger ones and some hatchery fish with these traits, crossed with wild fish, lay eggs that are intermediate in size.

Perhaps most surprising and significant is that similar biological effects have also been documented between wild Chinook salmon and hatchery-raised cohorts within a *single* generation. Hatchery fish are raised to fry stage from eggs taken from artificially spawned wild adults and released back into their natal river as a method of supplementing wild stocks. A report on levels of predation by "plunge-diving" Caspian terns (*Hydroprogen caspia,* which forage at or near the surface of the water) on juvenile salmonids in the Columbia River estuary, Oregon (USA) concluded that hatchery-reared smolts of Chinook salmon are more vulnerable to tern predation because they tend to travel in the upper water layers of the river, while wild smolts are more evenly distributed in the water column (Collis et al. 2001). Hatchery-raised fish of many salmonid species have been shown to be less responsive to all forms of predation than wild fish (Gross 1998; Jonsson et al. 1991) and this trait has been shown to be passed along to offspring of crosses with wild fish (Unwin and Glova 1997). Clearly, the effect of simply raising salmon eggs to the fry stage in captivity, with no intention of changing the population, can initiate biological changes that are detrimental to survival in the wild. This suggests

that significant and irreversible protodomestication changes can indeed be manifested within a single generation.

3.24 Discussion

If protodomestication was a natural speciation process initiated by the animals themselves, what prevented subsequent interbreeding with the source population? In other words, how do so-called "isolating mechanisms" develop in primitive domesticates if people did not deliberately isolate potentially interbreeding individuals from each other? I suggest that a significant shift in the timing of reproduction in early primitive domesticates must have contributed to the cohesion of the domesticated group, similar to the kind of reproductive timing shift experienced by Belyaev's foxes discussed in Section 1.4. These modern domestication experiments (Belyaev 1979, 1984; Hemmer 1990; Trut 1997) also provide evidence that after several generations of isolation and selection for non-fearful behaviour, really dramatic declines in fearfulness would have occurred concurrent with changes in reproductive timing.

The potential for such marked changes in behaviour during the early stages of protodomestication suggests that docile primitive dogs (and especially their young) might actually have sought out human contact. Coat colour differences that arose as a consequence of the process, such as white spotting (piebald) and solid colours (*non-agouti*), would have clearly differentiated primitive dogs and other domesticates from their wild cohorts after relatively few generations of separation. Given these physical, physiological and behavioural changes, the shift to an active relationship between primitive domesticates and people seems almost inevitable.

More docile behaviour might also have discouraged primitive domestic males from leaving during the breeding season, since wild females (e.g. of wolves, as well as other taxa with close-related domestic forms) are generally unwilling to accept domestic males as mates (Cronin et al. 1995; Friis 1985; Hemmer 1990; Lehman et al. 1990; Mengel 1971; Polziehn et al. 1995). Wild males, however, may still have approached primitive domestic females to breed, so that some introgression of wild genes (through the male lineage) may well have occurred in all taxa on a continuous, if irregular, basis. Protodomestication appears to be essentially irreversible, however, after the physical changes have occurred (even with occasional introgression) because "domestic" phenotypes (once they appear) are generally inherited in dominant fashion over the wild phenotype (Belyaev 1979; Friis 1985; Mengal 1971; Scott et al. 1959).

Once protodomestication has occurred, the new species (with its more docile behaviour and longer juvenile imprinting period due to paedomorphic changes) is one that could have been subjugated and managed by people with relative ease. This is the point at which all of the cultural influences traditionally described as "domestication" begin to uniquely shape the history of primitive domesticates (Figure 3.1, stage 4). Herbivores may have been corralled in order to keep them out of gardens and grain fields rather than to prevent them from "escaping," at least in the early stages of cultural control (dogs were rarely confined on a regular basis until quite recently, as I discuss elsewhere (Crockford 2000b). A more intense symbiotic relationship would gradually become established as people took varying amounts of control over living conditions of the animals, such as supplying food, shelter, and/or water.

Slight individual variations in TH rhythms must have continued to exist within

primitive domestic populations (and probably increased over time as natural mutations in the genes controlling these rhythms accumulated) and this provided the raw material for both natural and human selection to implement further change. Some distinctive morphotypes eventually developed as a result of continued selection for traits linked to heterochronic (paedomorphic) processes. The intensely juvenile physical and behavioural features of some domestic breeds discussed previously reflects the continued impact that heterochrony has had on domestic taxa subjected to artificial selection (e.g. Coppinger and Schneider 1995). The proposition that some breed variation is hormonally controlled through TH metabolism is supported by recent evidence that some dog morphotypes (e.g. sighthounds) have distinctly lower TH levels than the average for dogs in general (Ferguson 1994). However, spontaneous mutations in other genes (such as dwarfism) have obviously occurred and have been perpetuated through controlled breeding (Alderson 1978; Thomson 1996; Wilcox and Walkowicz 1989).

Some traits, however, cannot be controlled by selection in this fashion and a prime example is the extra digit (dew claw) in giant dog breeds. As Alberch (1985b) so eloquently explains, development of an extra digit is size dependant. Continued selection against this trait by breeders of St. Bernard dogs has not eliminated the extra digit because breeders also select for large size. Small dogs, such as poodles, do not develop extra digits because they do not reach the critical size required. Such relationships between seemingly unrelated traits (Müller 1990) impose distinct limitations on the forms which even intense artificial human selection can produce.

The underlying physiological interactions between the traits which change predictably as a result of protodomestication suggest that an entirely natural, biological

explanation is both plausible and testable, which I discuss in more detail later (Section 3.4). If "non-fearfulness" or "low anxiety" in relation to people can be considered a specific manifestation of the more general characteristic of "high stress tolerance," then the process of protodomestication can be seen as an entirely natural process; it did not necessarily involve direct interference by humans in the early stages, just their proximity. In other words, while the hypothesis does not preclude the deliberate actions of people in the initial stages of protodomestication, it does not *require* such interference (one could propose, for example, that Neolithic people might have observed a connection between non-fearful behaviour and certain physical characteristics in wild progenitors, such as specific coat colours or the position of facial hair whorls – as noted by Lanier et al. (2001) in domestic cattle - which would have allowed them to deliberately select non-fearful/stress-tolerant individuals out of large groups of animals, even newborns, without requiring any specific individual to demonstrate non-fearful behaviour at the time the selection was being made. If this were the case, a founding population of only non-fearful individuals could conceivably be created by deliberate intent that would be indistinguishable in ultimate results from natural protodomestication).

I propose that individual variation in TH rhythms among incipient domesticates provided the raw material for selection pressure to implement directed heterochronic change not significantly different from the process that changes incipient species into primitive new species. It is only *after* the protodomestication event occurs (Figure 3.1, stage 4) that cultural influences impart a distinctive history to primitive domesticates that distinguish them from other species.

3.3. The origin of species and adaptation

The intimate role that THs play in the response of animals to stress is pivotal to this concept. When populations expand their boundaries, new habitats offer many attractive benefits but they also present stressful conditions for each colonizing individual. Novel habitats are postulated as preferentially attracting physiologically stress-tolerant individuals (those with particular THR phenotypes) over less stress-tolerant animals. As colonizers constitute only those individuals with stress-tolerant physiological phenotypes, founder populations possess a non-random subset of the THR phenotypes (and the genes that produce them) maintained by ancestral populations. Variation of THR phenotypes (and the genotypes they represent) within such small founder populations will always be much smaller than that which existed in the ancestral population as a whole.

Due to the essential developmental role of THs, there would almost certainly be immediate consequences to offspring that result from mating within small and isolated founding populations that have limited variation in THR phenotypes. Rapid changes in morphology would be expected to occur in descendant populations as a new THR norm for the group becomes established. Most importantly, changes to behaviour and reproduction would occur simultaneously as the entire hormonal cascade is impacted by the THR shift. Under these circumstances, colonizers of new habitats become morphologically, reproductively, and behaviourally distinct so rapidly that they retain a very close genetic relationship to their ancestors. Nevertheless, a descendant population resulting from such a colonization event represents a new species as soon as a new THR equilibrium becomes established and once this speciation change occurs, it is permanent

as long as reproductive isolation is maintained. Behavioural and reproductive timing changes that occur as a consequence of the THR shift, in addition to the ecological partitioning that precipitated the event in the first place, tend to keep the new population reproductively discrete.

There is no evidence so far to suggest that any mitochondrial DNA haplotypes are associated with particular THR phenotypes. Thus by chance, even a small colonizing population of stress-tolerant individuals may possess all (or nearly all) of the mtDNA variation that existed in the ancestral population. This explains many examples of the extremely close mtDNA relationship of many taxa that by all other measures are considered fully distinct species. Such is the case for the polar bear, discussed in more detail below.

As stated previously, stress is an essential feature of virtually all speciation events. Stress is a ubiquitous factor that can have purely physical manifestations (such as light or temperature), or involve a psychological or behavioural component (such as dealing with predators, new food sources or competing for breeding sites). Existing variation in physiological or behavioural tolerance to stress could thus lead to the non-random subdivision of any population in response to changes in any stress-inducing condition.

After initial colonization events separate a few stress-tolerant individuals from an existing population, the particular circumstances of each new habitat present specific challenges to the founding population. Such challenges would impact the first few generations of founders heavily, leaving only those able to stay healthy enough to reproduce successfully as contributors to the next generation. The resulting changes in the descendants will vary according to the physiological makeup of each colonizing

population, its size and the severity of the selection pressure. However, this concept supplies the first really plausible explanation for how multiple speciation events (taxa with polyphyletic origins, or so-called "sibling species" (Mayr 1982; 1991)) could occur: given virtually identical ancestral populations (such as populations of geographically isolated subspecies) and similar or identical new habitats available for colonization, virtually identical biological changes would be expected to occur. If the geographic subspecies in question have been isolated long enough for them to have developed some level of genetic distinctiveness, the species each population generates will be genetically distinct despite the extreme similarities in morphological, behavioural and life history traits. The evidence is very strong that this is precisely what has occurred in the evolution of sea lions (Otariidae): northern and southern representatives each appear to have descended from northern and southern fur seal species, respectively (Lento et al. 1997) rather than from a single sea lion ancestor that later radiated north or south.

Similarly, the concept explains how convergent evolution could easily occur. When similar species are exposed to very similar climatic or habitat changes, we would expect similar kinds of changes to result because the thyroid control mechanisms are the same and would respond in a similar manner. The resulting organisms will be genetically distinct but similar in morphology – in some cases, the similarities are so strong that they make sorting out evolutionary relationships extremely difficult. Numerous examples of these phenomena are now known (e.g. Arnason et al. 1995) and the list is expanding rapidly. Similar ontogenic responses, such as the changing body sizes of mammalian taxa during the Pleistocene and Holocene (Kurtén 1988), could have occurred across taxonomic groups because both the stressor and the stress response system would have

been the same for all taxa (in much the same way that protodomestication produces paedomorphic changes in every instance).

As a consequence of the interaction between THs, sex and growth hormones (see discussion Section 2.2), heterochrony can also generate both individual and intraspecific variants (such as species-specific and sex-specific morphs), as well as interpopulational and interspecific differences, and it can accomplish these results both slowly and rapidly. While the concept does not explain *why* females are the larger sex in some taxa while males are larger in others, it does explain how such a pattern can be maintained as a species-specific trait. In addition, because development is a process that involves embryonic, fetal and post-natal programmed growth that is TH-dependent, heterochronic changes can be implemented at all ontogenic stages. Finally, colonization of radically different environments may often have necessitated quite dramatic dietary shifts that involved adding or deleting exogenous TH sources, factors that appear to be significant in the first two of the three examples I discuss below.

3.31 Origin of the polar bear

The first example of speciation mediated by heterochronic processes in natural (as opposed to anthropogenic) environments is demonstrated by the polar bear. Molecular genetic research by Talbot and Shields (1996a, b) on brown bear (*Ursus arctos*) populations in Alaska revealed surprising similarities between several mitochondrial genes of the polar bear (*Ursus maritimus*) and one particular coastal subspecies of brown bear. While polar bears had previously been proposed as descending from brown bear stock based on morphological criteria (Kurtén 1988; Kurtén and Anderson 1980), the

results of the genetic study place this one particular population of *Ursus arctos* as a sister species to *U. maritimus*. Although albino bears are known in many populations (Billingham and Silvers 1961; Banfield 1974; Lynch 1993), polar bears have dark eyes and noses (Searle 1968), a colour scheme that is technically extreme piebaldness (Barres et al. 1994). Talbot and Shields (1996b) themselves conclude that "..the morphological features distinguishing polar bears from brown bears have evolved rapidly in response to selective pressures of adapting to a new environment, prior to the emergence of distinguishing molecular features."

I suggest that isolation of brown bears in restricted glacial refugia at some time during the late Pleistocene, probably in Siberia or SE Alaska, created the conditions that encouraged a few stress-tolerant individuals to colonize the pack ice environment surrounding these refugial "islands." Such an environment would have been rich in animal food resources –especially seals – foods rarely available to animals in the original source population (although some brown bears in the northern extremes of their range are known to kill and eat seals, as Lynch (1993) and Struzik (2003) have noted). I propose that a few stress-tolerant brown bears (those with particular THRs), colonizing pack ice where foods high in exogenous THs (seals) were all that was available, were exposed to virtually perfect conditions for rapid speciation to occur (see also Crockford 2003, Appendix B; figure 3).

Profound changes in morphology and physiology, including the generation of a distinctive piebald coat colour, would have been inevitable within only a few generations under these conditions. It may have taken many generations for the extreme piebaldness characteristic of modern polar bears to dominate the population, but a less extreme black

and white coat must have been the present even in the earliest animals. If being completely white gave polar bears a survival or reproductive advantage, partially piebald animals may have been selected against until only extreme ones remained (as was done with Samoyed dogs, which transformed via human selection from a black and white animal in that late 1800's to a completely white dog less than 100 years later; Wilcox and Walkowicz 1989).

If polar bears possess extreme piebaldness as a consequence of the rapid speciation changes precipitated by colonization of a radically new environment, then perhaps other white arctic taxa (such as hares, foxes and wolves) and white alpine species (such as mountain goats) are white for this reason as well (Banfield 1974; Chesemore 1975; Jolicoeur 1975; Geist 1971, 1986; Searle 1968). Being completely white in arctic or alpine environments may have conferred an immediate survival advantage, and partially piebald animals, as for primitive polar bears, may have been heavily selected against until only extreme piebald animals remained.

The essential point, however, is that piebaldness could only have become subject to selection pressure because heterochronic speciation changes made it an available phenotype. Piebaldness is normally far too rare in most wild populations for selection alone (especially in top predators like the polar bear that have no or few natural enemies) to have created a whole population of extreme piebald animals (even over a very long period of time) unless something occurred to increase the natural incidence of this anomaly substantially. Belyaev et al. (1981), for example, calculated the gene frequency for the *piebald S* gene in two control, or unselected, populations of silver foxes as 1.1×10^{-2} and 7×10^{-3}, while the frequency in the population selected for "non-fearful"

behaviour increased to 3.7×10^{-2}.

This suggests that the selective pressures on animals whose normal colour is a striking black and white pattern (e.g. skunks, the giant panda, killer whale, etc.) probably have not been exposed to selection as extreme or intense as animals that are completely white (e.g. polar bear, beluga whale, mountain goat). Just because some animals have successfully dealt with being conspicuously piebald does not mean that piebaldness in and of itself confers any kind of adaptive advantage, as is usually suggested (e.g. Morris and Morris 1966; Searle 1968). On the contrary, behavioural traits controlled by physiological factors could have conveyed the initial adaptive advantage for the ancestors of piebald taxa and piebaldness may simply be an inevitable consequence, an artifact or "spandrel" (Gould 2002), of small populations of physiologically-similar individuals colonizing radically new habitats. If so, piebaldness as a normal species trait may represent a significant indicator of major habitat shifts associated with heterochronic speciation in the history of those taxa.

3.32 Origin of hominid bipedalism

Another example of interest is hominid evolution, a lineage for which a case can be made for THR involvement in both species-level and genus-level heterochronic changes. Although I discuss this topic in depth elsewhere, proposing a possible TH-mediated scenario for several speciation events throughout the entire five to six million year history of the hominid lineage (Crockford 2003, Appendix B), I summarize here only my discussion of bipedalism (two-legged locomotion).

Bipedalism is the earliest hominid trait noted in the fossil record: the particular

pelvic, vertebral and femoral shape changes that allowed upright stance and locomotion preceded other morphological characteristics (like a larger brain, shorter gut, larger body size, smaller teeth) that make later hominids unique (Fleagle 1999). What could have initiated such particular morphological changes in the first place? Various suggestions have been advanced, most of which assume that bipedal morphology arose because it conferred some survival or reproductive advantage (such as heat dispersal (Wheeler 1984, 1991), carrying infants, or using tools (Jablonski and Chaplin 1992) - discussed recently and in depth by Kingdon (2003), Leonard and Robertson (1997) and Leonard (2003).

However, you do not get bipedal morphology - or a bigger brain and shorter gut, as per Aiello and Wheeler (1995) - because they would be advantageous to survival. Evolution is not a mail-order catalogue: natural selection can act only on traits that are already present in a population (Mayr 1982). Just as for piebald phenotypes, this means that some incipient hominids had to possess bipedal morphology while others did not.

The skeletal traits associated with bipedal locomotion have been demonstrated to be heterochronic in nature (Berge 2002; Carroll 2003; Parker 2002), although these do not appear to be the same heterochronic changes that later generated larger brains, as Lovejoy et al. (1999) point out in their discussion of bipedal anatomical development. This suggests strongly that a significant and rapid heterochronic speciation event precipitated the changes associated with bipedalism, a speciation event that I suggest was almost certainly associated with colonization of a very distinctly different habitat. Not surprisingly, the geological period concerned (ca. 5-7 mya) shows ample evidence that dramatic change was underway in Africa (e.g. deMenocal 2004; Hill 1994; Kelly 2002; Kingdon 2003; Potts 1998; Reed 1997), although the exact nature of those changes in

some locales is uncertain.

I suggest, quite simply, that the first Australopithecines (presuming these are indeed the first bipedal hominid ancestors, which is not universally accepted) evolved with a novel bipedal morphology because some of their ancestors chose to colonize a habitat in which the prevalent foods were not the fruits they were accustomed to eating but small animals: bird eggs and fledglings, small mammals, reptiles and amphibians (supported by isotope evidence, Sponheimer and Lee-Thorp 1999). Such a dietary change would have been especially profound because it involved the consumption of vastly increased amounts of exogenous THs. Small prey animals such as rodents, reptiles, amphibians and young birds are generally eaten whole, which means their thyroid glands and livers (which contain especially high concentrations of THs) are generally consumed as well (Eales 1997). Egg yolks of all taxa also contain THs (McNabb and Wilson 1993).

As stated previously, THs are the only hormones that are readily absorbed unaltered through the digestive tract; exogenous THs from animal prey are indistinguishable from self-produced hormone and present in concentrated form. Herbivores or fructivores, even if they occasionally ate small animals, would have possessed a TH metabolism unprepared for such excess. Consumption of large quantities of TH-laden foods (rather than occasional small amounts), day after day and month after month, would have had a major impact on populations of incipient bipedal hominids.

I suggest that only those individual hominid ancestors who were relatively tolerant of high stress situations would have chosen to colonize a radically new environment in the first place. Experimental domestication suggests this stress-resistant component could comprise as much as 20% of the existing population (Belyaev 1979). Even stress-tolerant

colonizing individuals, however, would have varied somewhat in their ability to accommodate a dramatic increase in exogenous THs from food resources without a major disruption of their reproductive potential.

I call this quality of responsiveness to exogenous THs "THR resilience" to distinguish it from the characteristic previously discussed, that of "stress tolerance." Furthermore, I suggest that evidence from cancer research supports the existence of this phenomena in modern humans. In two recent clinic trials, researchers attempted to induce biochemical hypothyroidism (free T4 <0.7ng/dL or TSH above 10 μ/L) in patients with malignant brain tumours (recurrent high-grade *gliomas*) in order to increase responsiveness to tumour-suppressing drugs – trials prompted by their previous work that that showed many cancers require *insulin-like growth factor-I* (IGF-1) for enlargement and thus are T_3 dependent (Hercbergs 1999). However, only about one half of patients in either study reported (18/34 and 11/22) responded as expected to TH-reducing drug therapy; the rest remained euthyroid (Hercbergs et al. 2002; 2003). These results confirm that even among small groups of modern humans with cancer, a significant amount of variation exists in the resilience of thyroid function to ingestion of substances known to reduce THs. Indeed, such underlying individual variation may well explain the frustratingly variable responses characteristic of virtually all cancer treatment regimes (Hercbergs, personal communication, 2002) and suggests strongly that responses to increases in blood THs levels, due to consumption of THs from exogenous sources, would vary similarly.

How would a dramatic increase in amounts of exogenous THs affect foetal development in a founding group of incipient hominids? There is abundant evidence that

exogenous THs cross the placenta (in both experimental animals and modern humans) and that significant changes in normal TH levels during pregnancy (either too much or too little) have profound affects on the developing foetus (Chan and Kilby 2000; Cudd et al. 2002; Porterfield 2000; Waterman 1958; Weetman 1997). I suggest there is no reason to expect that incipient hominids would have responded differently to levels of exogenous THs that far exceeded their normal intake.

I propose (based on the known requirement in all vertebrates of appropriate levels of THs at appropriate times in maintaining a normal pregnancy and for embryonic development and foetal growth) that a major shift in diet such as I have suggested for founding groups of incipient hominids, would probably have resulted in some instances of reduced fertility (failure to ovulate or conceive, repeated miscarriages or stillbirths) and a relatively high incidence of birth anomalies of various kinds (as reported by Waterman 1958 for high exogenous doses of THs given to pregnant rats). Live offspring afflicted with profound anomalies probably died young. However, survival rates of infants with relatively minor anomalies may have been quite high and among these could have been a suite of slight change in pelvic, vertebral and femoral shape that allowed offspring to stand upright with ease. As long as such anomalies did not negatively impact the survival of afflicted individuals, those offspring would have had a reasonable chance of living to sexual maturity and passing on their genes to the next generation.

A possible modern analogue of this scenario exists in another primate, although the underlying causes are somewhat different: the minor birth anomalies long known in some populations of Japanese macaque, *Macaca fuscata,* (possibly caused by chemical contaminants in provisioned food) are clearly mitigated by the determination of some

mothers to raise afflicted offspring regardless (Turner 2003). Some afflicted offspring not only survive with truly crippling anomalies but are reproductively successful. One well-known female lived for 26 years and raised five offspring despite severe defects in both hands and feet. This on-going natural experiment suggests that the behavioural response of mothers to offspring with relatively minor physical birth anomalies in my scenario for bipedal hominids is possible for a primate.

In summary, I suggest that offspring with new viable morphologies were generated immediately in founding groups of incipient hominids because their mothers possessed the genes for a particular THR phenotype that was resilient to a diet high in exogenous THs. This "resilient THR phenotype" allowed almost-normal foetal development to proceed, generating offspring with relatively minor morphological differences, probably accompanied by some behavioural and reproductive physiological differences as well (based on what we know about the nature of TH-mediated heterochronic changes). If bipedal offspring had a THR phenotype similar to their mother, they may have been more likely to produce bipedal infants themselves when consuming a similar diet. Over the next few generations, the specific growth programs capable of producing bipedal morphology would have increased in frequency until they become the norm for the whole population. Colonization of a radical new habitat and the associated dietary switch was responsible for precipitating the rapid expression of several new morphotypes but natural selection was responsible for the fact that bipedalism was the option that perpetuated. Disruptive levels of exogenous THs are probably not responsible for producing the bipedal morphology themselves; rather, they likely provided an enduring selective factor which reduced the breeding population of colonizers, in each

succeeding generation, to only those females with a THR resilient enough to produce viable offspring under prevailing conditions of high exogenous THs.

However, I suggest there is also a possibility that disruptively high levels of exogenous THs could have permanently altered the phenotype of the newborn offspring and altered its neonatal growth program, through impacts on the foetal SCN clock genes that control the THR. If timing and absolute amounts of THs are critical to the development of embryonic brain cellular architecture, as appears to be the case (Garcia-Segura and McCarthy 2004; Lavado-Autric et al. 2003), disruptive amounts of exogenous THs could have impacted maternal THRs to such an extent that normal embryonic migration, proliferation and maturation of SCN oscillating cells were slightly compromised (slight differences in the physical relationship of these neurons to each other might slightly alter their combined output just enough to produce THR differences in offspring capable of permanently affecting the growth, development and future behaviour; see also discussion in Section 3.33). It is possible that disruptive levels of exogenous THs impacted founding populations of early bipedal hominids by providing strong selection on maternal THRs *and* through effects on maternal THRs that generated slight but permanent changes in foetal SCN architecture.

An exogenous TH explanation for the rapid generation of macroevolutionary bipedal morphology raises the question of whether bipedalism is exclusive to the hominid lineage. Claims of bipedal morphology (either by direct fossil evidence of post-cranial material or inferred by cranial characters) have been made for *Ardipethecus ramadens* (4.4 mya, Fleagle 1999; White et al. 1994), *Australopithecus anamensis* from east Africa (ca. 4 mya, Ward et al. 2001; Leakey and Walker 2003); *Orrorin tugenensis* (5-6 mya,

summarized by Lieberman 2001), a controversial recent find, *Sahelanthropus tchadensis* (6-7 mya, summarized by Wood 2002) and the so-called "swamp ape" from Sardinia, *Oreopithecus bambolii* (7-9 mya, Kohler and Moya-Sola 1997; Rook 1999). If some or all of these hold up, we may not only have to revise the earliest date for hominid origins (Stringer 2002) but acknowledge that bipedalism is not exclusive to this lineage.

The pertinent question is which of these bipedal genera are ancestral to the line that produced later *Homo sapiens* and which specimens represent cases of convergent evolution (e.g. Wood 2002)? An arid climatic period near the end of the Miocene (ca. 6.2 mya, and an earlier one at ca. 7.8 mya) resulted in a marked reduction in the variety and abundance of fruit-bearing trees across East Africa (Kingdon 2003:121), suggesting that more than one ape lineage may have been forced to assume a diet heavily dominated by small animals (a conclusion Kingdon himself comes to, but without realizing what the developmental consequences would have been for vegetarian primates). Consumption of high levels of exogenous THs would have had similar effects on any ape population as that described for Australopithecines: not identical, but similar enough to cause us confusion in sorting out the scanty fossil remains of closely-related lineages. Just because *Orrorin* was bipedal doesn't automatically make it a direct human ancestor, although the same could also be said for *Australopithecus*. Sorting them all out will take many more years of work and more quality specimens, but the THR concept (providing a mechanism for evolutionary change to be rapid on occasion rather than always slow), makes possible a number of new options in the interpretation of fossil evidence that did not exist before.

What about speciation events subsequent to the generation of bipedalism in the hominid lineage? Many authors have taken the same basic approach used with bipedalism

to try to explain the acquisition of other hominid-exclusive traits, but no matter how sound the scientific basis of their arguments (e.g. Aiello and Wheeler 1995; Leach 2003; Horrobin 2001; Kingdon 2003), without a biological mechanism to explain *how* these traits could be generated by natural selection, all become "just-so" stories. In contrast, I have been able to use the THR theory to suggest, in explicit biological terms, how particular speciation events in the hominid lineage could have generated particular heterochronic traits, including unique Neandertal morphotypes (Crockford 2002b, Appendix A; Crockford 2003, Appendix B; Minugh-Purvis and Crockford 2002). In the hominid lineage particularly, I have proposed that dietary changes associated with colonization of new habitats involved shifts back and forth in relative amounts of exogenous T_3 and T_4; these habitat-plus-diet-shifts probably drove all but the most recent modifications, which appear to have been entirely diet-driven (see Crockford 2003, Appendix B; figure 5).

The suite of morphological characteristics that emerged over the course of hominid evolution (including, but not limited to, increased linear stature, increased cranial capacity and brain complexity, pelvic and femoral changes, shortening of the gut, and slowing of tooth eruption) involve heterochronic change (e.g. McKinney 1998, 2002; Minugh-Purvis and McNamara 2002) and thus are potentially explainable by the effects of shifting proportions of THRs in populations (see Crockford 2002b, 2003; Minugh-Purvis and Crockford 2002). Due to the fact that THs exert considerable control over hair and skin growth as well as its colour, dietary shifts that impacted developmental programs even slightly could have affected many seemingly unrelated traits as a consequence, such as skin colour (cf. Jablonski and Chaplin 2000, 2003). Indeed, two distinctive features of

modern humans that have been especially hard to explain as selected traits are our unique pattern of fat accumulation (especially the production of "fat" neonates, as discussed by Horrobin (2001), and our relative hairlessness. I suggest these may also have been inevitable consequences of rapid heterochronic change that took place in the hominid lineage, perhaps quite early on, and thus were never actively selected traits themselves (cf. the suggestion made by Wheeler (1984) that hairlessness provided a selective advantage related to thermoregulation).

Fluctuating diets, both associated with habitat change and independent of them, may have been instrumental in maintaining a large pool of THR variation in the hominid lineage, variation ultimately essential to the success of our species. The innovation of using fire to cook meat appears to be the stimulus that finally pushed our ancestors over an important evolutionary line, creating modern human morphology as a consequence of a diet change alone (see also Crockford 2003, Appendix B). Changing the TH constituent of an established diet simply by preparing it differently (as opposed to making it more tender or digestible, as suggested by Wrangham and Conklin-Brittain 2003), although not previously considered before now, is something only hominids have done. Therefore, using fire for cooking (rather than for light or heat) may have been responsible for making modern humans the truly unique species we are – although certainly the ability to make and use tools in the first place gave our ancestors distinct choices in food and life history strategies that helped set the path of their biological evolution.

3.33 Fluctuating adaptation in Darwin's finches

I have so far addressed the issues of microevolution and macroevolution almost

exclusively. However, the concept presented here also explains both fluctuating and permanent adaptation of established species over time. Because new conditions can be imposed on an entire population - by sudden climatic shifts, for example – I suggest that only those individuals who already possess the traits (controlled by underlying THR phenotypes) that allow them to survive the changes and reproduce successfully despite them, pass their genes on to future generations. Thus, some THR phenotypes may be reduced to only a few representatives in one generation because most individuals with that THR phenotype die or do not reproduce, leaving only alternative THR phenotypes to contribute to the next generation.

An example of how rapidly adaptation can occur, and that may well represent an example of TH-mediated changes, has been reported recently by Grant and Grant (2002). These researchers undertook a rare long-term investigation of two species of finches from Daphne Major in the Galapagos, the medium ground finch (*Geospiza fortis*) and the cactus finch (*G. scandens*), between the years 1972 and 2001. In their study, changes in the food supply over a 30-year period (brought about by alternating periods of drought and flood) selected several times for beaks of significantly different shapes and sizes, sometimes accompanied by changes in overall body size.

If beak shape and size, as well as body size, are traits set during early development and thus controlled by species-specific THRs (with slight individual variations), as I propose must be the case, changes in food supply could alternately select for beaks of one size and/or shape (accompanied in some cases by body size shifts) according to the size of the most prevalent seeds available in different years. If so, this case demonstrates that even over the short term, individual variation in THR phenotypes

could provide the essential plasticity for a population to shift rapidly in response to changes in environmental conditions so that overall population survival is ensured.

The authors of this study also found evidence, during the later ten years of the study, of introgressive hybridization between the finch species – hybridization that appears to have increased the variation in beak size and shape available to both species without negatively affecting the fitness of either. These effects of hybridization raise an important question: if variation becomes much reduced in an adapting or colonizing population, how does it later increase so that adaptation can continue in the future? Obviously, hybridization with a closely related species is one way to add variation back into an otherwise restricted gene pool.

It may be possible, however, that the genes controlling THR phenotypes in oscillating SCN clock neurons (as described earlier in Section 2.7), accumulate mutations at a fairly rapid rate, quickly replacing variation lost during a population crash. Given that there may be many genes whose combined actions generate THR phenotypes and that each individual gene within that complex cannot be subject to selection directly (since it is their combined product – the neural or neurohormal output, including the THR - that is selected via its down-stream affects on physiological and physical traits), the individual genes that produce THR phenotypes may undergo slight mutations in virtually neutral fashion (with no single genetic variant either advantageous or disadvantageous on its own), creating a multitude of alleles virtually continuously. Such mini-mutations would ensure that individual differences in THRs are constantly replenished. This is pure speculation, of course, but worth checking once the suite of genes that produce THRs have been confidently identified.

Alternatively, given the critical requirement for THs in all vertebrates in generating fine-scale neural architecture of the brain in a dose and time-dependent manner (e.g. Zoeller 2003; other references cited in Part 2) and evidence that an intact maternal SCN is required for normal development of SCN rhythms in newborn rats (Reppert and Schwartz 1986), another explanation for continually renewed variation presents itself. Rather than the genes of pertinent SCN oscillating clock neurons changing, perhaps it is the physical relationship of those cells to others in the clock complex. I suggest the possibility exists that minute shifts in such physical inter-relationships (due to slight variations of THR during brain development), might result in minor differences in rhythmic output. Although a comparative analysis of the SCNs of two mouse strains with distinct circadian rhythms has been undertaken (Lippa et al. 1992), only gross anatomical features were investigated (i.e. SCN length, girth volume, shape and cell counts); no assessment was made of the kind of cell-to-cell positional differences that I propose may be significant. If THs regulated by individually-unique THRs of the dam modulate neural cell migration, proliferation and maturation (at least in placental mammals), as I suggest must be the case, such slight differences in the relationship of critical SCN oscillating neurons to each other might ultimately produce distinct THRs in offspring.

Such maternally-mediated effects on SCN architecture represents a possible mechanism to explain an intriguing phenomenon only recently disclosed by researchers working on the new technological process of "nuclear transfer" (often colloquially, but erroneously, called "cloning"). Using genetically identical cells implanted into a surrogate mother or mothers, this technique has so far failed to produce truly identical offspring

(Ezzel 2003). For example, Ezzel cites work by T. Friend and G. Archer (Texas A & M University) which demonstrates this effect on pigs: among the two litters of pigs produced (consisting of four and five offspring each) by implantation of genetically identical embryos into two different surrogate sows, there was as much individual variation in physical and behavioural traits (e.g. in coat "bristliness", number of teeth, food preferences, temperament) as within two natural pig litters. Similarly, in cattle (Lanza et al. 2001), the 24 healthy cows generated so far by implantation of genetically-identical embryos into different surrogate mothers are described as displaying normal differences in temperament. Social hierarchies have become established in the "cloned" herd that are typical of those established in herds of naturally-produced cows; in addition, the ages given for attaining sexual maturity in cloned cows also varied within the range exhibited by naturally-produced offspring (i.e. 10 to 12 months). The researchers interviewed by Ezzel suggested only differential conditions within the womb (for litters of pigs) and unspecified *epigenetic* effects (differences in the interactions of genes during development) as possible explanations for their results. However, I suggest my theory for the role of individually-unique maternal THRs in foetal development not only explains these phenomena more precisely and completely but is an eminently testable option.

3.34 Discussion

If THRs are indeed responsible for keeping the individual adapted to environmental conditions that change on a daily and seasonal basis, as I propose must be the case, this physiological system has the potential to initiate and implement the changes

that allow species to adapt over longer periods of time. The range of variation for THRs possessed by any species undoubtedly influence its evolutionary adaptability. We would therefore expect some species to be better able to adapt to specific kinds of environmental change because their populations are composed of individuals with more physiological, morphological, and behavioural variation. In contrast, some sets of quite distantly related species could all change in a similar fashion on exposure to identical shifts in environmental regimes over evolutionary time (such as extreme low or high temperatures). Similar ontonogenic responses, such as the changing body sizes of mammalian taxa during the Pleistocene and Holocene (Kurtén 1968, 1988; Kurtén and Anderson 1980; Smith et al. 1995), could have occurred across taxonomic groups because both the stressor and the stress response system would have been the same for all taxa (in much the same way that protodomestication produces paedomorphic changes in every instance).

As a consequence of the interaction between THRs and sex and growth hormones, heterochrony controlled by THRs can generate both individual and intraspecific variants as well as interpopulational and interspecific differences, and it can accomplish these results both slowly and rapidly. In addition, as development is a process that involves both embryonic and postnatal programmed growth dependent on THs, heterochronic changes controlled by THRs can be implemented at all ontogenic stages rather than constrained to embryonic effects as suggested by Raff (1996).

Is this paradigm applicable only to vertebrates? Taken in its broadest sense, I suspect not. Gibson and Hogness (1996; summarized in Tautz 1996), for example, have presented experimental evidence for *Drosophila* that repeated selection for a

physiological phenotype combined with small population sizes affects developmental processes. In their study, flies selected for sensitivity to ether produced, over several generations, descendants with bithorax anomalies (four wings) indicative of developmental disruption. These results, along with a recent overview by Whiting et al. (2003) on evolutionary patterns of loss and recovery of wings in stick insects, suggest that a physiological mechanism controlling development, similar to that proposed for vertebrates, may also exist for invertebrates. The controlling substance in insect may be *juvenile hormone* (JH), a molecule very similar in structure and developmental functions to retinoic acid (Adám et al. 2003); certainly, Nijhout's (1999) overview of JH and polyphenic development in insects would support such an assumption.

The same may be true for plants, which also possess hormones whose interactions affect growth and development (Farnsworth 2004; Hoffmann and Parsons 1991; Klee 2003; Langridge 1991), and for which clear rhythmic cycles are evident (Sweeney 1969). Heterochrony has been shown to operate as a significant evolutionary process in many plant lineages (Guerrant 1988; Iltis 1983; Lord and Hill 1987). The mechanism controlling this process in plants is still a mystery despite concerted research efforts - including the recent addition of molecular genetic studies (e.g. Kim et al. 2003).

Surprisingly, however, a number of species of kelp and other algae, long known to be rich sources of iodine, were found to store much of this essential mineral as T_3 and T_4 (Johnson 1997), providing ready-made THs to all animals that consume them – whether they have backbones or not. THs have also been found to be produced or utilized in a range of marine invertebrates (Berg et al. 1959), including corals (Kingsley et al. 2001), and echinoderms (Chino et al. 1994; Johnson and Cartwright 1996; Johnson 1998).

Although THs have been shown to control or modify development in these organisms – as well as in some insects and plants – mechanisms of production and control of THs are less complex than in vertebrates. In many organisms, for example, THs utilized during development may come exclusively from exogenous sources. In this respect, as noted by Eales (1997), THs function more like a vitamin than a classic hormone. As Eales emphases, THs are similar in this respect to vitamin D, a molecule that in many higher organisms can either be extracted from exogenous sources (as a vitamin) or manufactured (like a hormone), while in less complex taxa they must extracted from food sources.

Thus, THs may have an exceptionally broad evolutionary role in growth and development that lies well beyond the model proposed here for vertebrates. Indeed, the similar developmental roles and molecular similarities - especially in essential binding site configuration - between THs, RA, and JH, are unlikely to be coincidental. The fact that plants contain a number of compounds that belong to the same family of terpenoid molecules as RA and JH (e.g. chlorophylls, carotenoids, gibberellins, and abscisic acid) (Bede et al. 2001), may also be significant.

3.4. Testing the hypothesis

This paradigm assumes that individual TH rhythm variants (THR phenotypes) exist within species-specific patterns for animal populations and that these THR phenotypes are the actual characteristics targeted by natural selection in instances of adaptation and colonization. It predicts that non-random subdivision of populations often occurs during speciation, isolating particular subsets of individuals with similar THRs within founder populations. Developmental repercussion of lowered THR diversity in the

founding group is assumed to be responsible for generating the heterochronic changes seen in descendant taxa.

The basic hypothesis to be tested, therefore, is that daily rhythmic TH secretion profiles of any vertebrate species are individually variable and that these variations between individuals can be correlated with discernable morphological, reproductive, and/or behavioural differences (THR phenotypes). As a group, individual THR phenotypes together should generate a distinctive pattern for the population that is species-specific (or in the case of domesticates, breed-specific): that is, the average pattern for the group should be distinguishable from that of a closely-related taxon.

Devising experiments that can reliably test this premise will undoubtedly be difficult due to the dynamic nature of the endocrine system and its inherent sensitivity to stress of any kind (Boissy 1995). Such sensitivity presents a unique challenge to the determination of normal THRs within and between taxa, since TH levels must be measured frequently (at least every 15-20 minutes, preferably every 5 minutes), under controlled conditions for many individuals.

However, Windle et al. (1998) have demonstrated that automated sampling may circumvent many of the difficulties of testing the THR hypothesis. For their study on corticosterone levels in rats, a surgically implanted cannula connected to an automated blood-sampling apparatus (illustrated in Lightman et al. 2000) allowed minute quantities of blood (10-20 μl) to be collected every ten minutes over a twenty-four hour period without disturbing the animals by repeated handling. The two breeds of rats they used showed significant differences in mean profiles of hormone production (as well as slight individual variations within breeds) in addition to significant differences in behavioural

responses to a controllable stress (i.e. white noise) (see Crockford 2002b, Appendix A; Figure 2). The success of these experiments in demonstrating the existence of fine-scale patterns of corticosterone production and in correlating these profiles to stress responses suggests that a similar method might be suitable for testing the THR hypothesis (similar automated sampling methods have also been applied to studies on pulsatile hormone secretion in humans (Adcock et al. 1997, Robinson 2000, Russell-Aulet et al. 2001, Veldhuis 2000, Lucke et al. 1977), fish (Gomez et al. 1997) and the domestic cat (Kemppainen and Peterson 1996).

However, using automated blood sampling devices for addressing the THR hypothesis for a broad range of taxa will not circumvent all potential testing problems. For example, current laboratory assay methods for measuring THs in minute quantities may place limitations on the smallest samples that can be analyzed – new assay methods may need developing (cf. Adcock et al. 1997). The sampling apparatus itself may need modification to allow testing of a full range of animals: laboratory-housed fish species and free-ranging bear species, for example, pose different logistical problems for an automated TH sampling and storage device.

However, if individual variation within species-specific profiles of THRs can be confirmed, controlled breeding experiments (similar to those described for silver foxes in Section 1.4) will be necessary to confirm that small interbreeding groups of physiologically-similar animals (i.e. with similar THR profiles) produce phenotypically different descendants in 20 generations or less. It would be most convincing if descendants of such breeding programs could have their THR profiles monitored as well, for the mean of these THRs should differ from the mean of the original source population.

If it can be demonstrated that THRs are indeed variable within certain limits for different populations (or breeds, or for certain morphs within species) *and* that heterochronic changes can be generated by interbreeding small groups of physiologically similar individuals, the final step will be to find the genetic sources of those pattern differences. Although species-specific THRs are probably controlled by genes in the SCN that are directly associated with generating hormone pulsatility, other factors may effect TH utilization in ways that are also species-specific. For example, Gagneux et al. (2001) have suggested that blood concentrations of transthyretin in chimpanzees and humans appear to be significantly distinct. However, I suggest that the first step in genetic characterization of individual and species-specific TH rhythm profiles should be documenting variation in SCN output. Certainly, genes encoding TH hormone receptors, receptor ligands and/or cofactors in target tissues are potential source of variation in TH-mediated phenotypes (e.g. Bassett and Williams 2003; Flamant and Samarut 2003; Russell et al. 2003; Yen 2003). However, it is expected that in most case, differences in such genes will be found to supplement, compound or confound THR effects rather than contribute to their initial rhythmic generation (and as a consequence, genes involved in such TH-mediated "end-factor" processes could be selected for independently from THRs or simultaneously, suggesting that mutations in such genes may explain the origins of some evolutionary novelties that are *not* heterochronic in nature).

In the mean time, on-going research into the regulatory mechanisms of embryonic development should unravel some of the essential molecular interactions that involve THs and THRs. Research on *Hox* genes that act during embryonic development has thus far revealed they respond to retinoic acid as well as other molecules (e.g. Lawrence and

Morata 1994). In light of the known developmental regulation functions that retinoic acid shares with TH, or in which their roles cannot be distinguished (Barres et al. 1994; Davis and Lazar 1992; Evans 1988; Schilthuis et al. 1995; Song et al. 1995; Stephanou and Handwerger 1995), it would be prudent for researchers to look at the response of *Hox* genes to THs, and/or THR pulses, in combination with retinoic acid. Given the critical roles recently demonstrated for THs themselves in embryonic development (e.g. Garcia-Segura and McCarthy 2004; Lavado-Autric 2003; Zoeller 2003), we also need to know what effects different THR profiles might have on any given developmental program.

Lastly, research into the physiological and genetic basis of natural piebaldness (rather than aberrant white spotting mutants) may also be illuminating. Piebaldness, if we can come to understand exactly what it signifies, could serve as an especially useful diagnostic marker for heterochronic change.

3.5. Summary and Conclusions

As Darwin may have suspected, understanding the process that produced domestic animals appears to hold the key to unlocking the mysteries of evolutionary change. I suggest that the process of *protodomestication,* as I have defined and explained it here, becomes an appropriate model for describing the truly dynamic relationship that exists between individual variation, adaptation and speciation in all vertebrate taxa, including humans.

I have tentatively identified individual variation in species-specific rhythms of TH production (THRs) as the essential factor linking individual variation in a wide variety of selectable traits, including morphological, physiological, reproductive and behavioural

characteristics. Using real species pairs as putative examples, I have proposed that during colonization events, non-random population subdivision isolates small groups of stress-tolerant phenotypes as founders, which inevitably initiates the developmental changes associated with heterochronic speciation in their descendants (e.g. wolf to domestic dog; brown bear to polar bear). Non-random population subdivision in other circumstances can be achieved via selective mortality for certain physiological phenotypes, again leading to heterochronic changes in descendants of founders (e.g. wild to domestic rainbow trout). Adaptation of entire populations to changing environmental conditions can be achieved over both short and long time periods via selection for any THR-determined trait, due to shifts in the relative proportions of THR phenotypes in the group (e.g. Darwin's finches on Daphne Major) - this adaptation can be temporary or permanent (resulting in speciation), depending on the circumstances. Reduced THR variation in small founder or adapting populations can be increased via hybridation with a closely-related species (e.g. Darwin's finches on Daphne Major), or via natural accumulation of THR variation over time (due to mutations in SCN clock genes or as a consequence of slight changes in the physical relationships of individual oscillating SCN clock neurons to each other).

A strong body of evidence supports the few assumptions I have had to make in the course of developing this theoretical model, which I have presented in detail in Chapter 2. In summary, there is strong evidence of pulsatile release in all hormones, including THs. In addition, all hormone rhythms are known to change in response to factors such as season and reproductive stage, and disruption of these rhythms are known to be associated with both illness and aging; active research programs are in place that continue to investigate the precise nature of the molecular mechanisms that generate hormone

pulsatility. Many critical roles for THs in embryonic, foetal and postnatal development (especially in brain formation) and in fundamental cellular and mitochondrial processes have been now been documented at the molecular level.

As for the issue of how much influence THs have over other hormones, the support is strong for most interactions but weaker for a few. Specifically, there is compelling evidence (characterizing interactions at the molecular level) that THs have a critical role in the secretion and actions of GH/IGF-I (*growth hormone* and its active analogue, *insulin-like growth factor-I*), from pulsatile release of growth hormone-releasing hormone from the hypothalamus through to tissue actions on developing bone and muscle; the same is true for adrenal hormones (glucocorticoids and catecholamines) and prolactin, a reproductive hormone produced by the pituitary gland. While there is ample evidence that the primary reproductive hormones of the gonads (testosterone and oestrogen) are intensely responsive to changes in TH levels, the precise nature of their molecular relationship is not well understood and continues to be an area of very active research. Over all, the literature presents a picture of very strong support for the interpretation that since an intimate relationship exists between THs and other hormones, and all hormones are known to be secreted in rhythmic fashion, pulsatile TH rhythms may constitute a critical hormonal pacemaker in all vertebrates. However, definitive evidence awaits research directed specifically at this question.

While the hormonal connections I have suggested should exist between behavioural responses to stress (including displays of social dominance and territoriality, as well as levels of relative fearfulness), daily THRs (including effects on adrenal glucocorticoids and catecholamines), and reproductive timing have not yet been explored

in a manner that supports my assumptions, a wealth of experimental evidence on *components* of this relationship already exists (for example see Fox et al.'s (1997) study on stress, dominance behaviour and reproductive success in the African ciclid fish, *Haplochromis burtoni,* Morgan et al.'s (2000) study on the effects of THs on estrogen-induced sexual behaviour in female mice, and Hardy et al.'s (2002) work on adrenal hormone levels vs. sex hormone levels in relation to social stress in male rats - as well as references therein).

As I have discussed in Chapter 2, although daily variations of TH concentration that result from pulsatile TH secretion have been known about for many years in humans, they have been assumed to have little or no impact on "normal" biological function (e.g. Luck 1977; Stockigt 2000). In contrast, recent work on rats suggests that very minor variations of T_4 concentration in the foetus alter the development of critical brain cell architecture (Zoeller 2003). Merging these research approaches would effectively test a corollary of the basic hypothesis that predicts variations in maternal THRs (in placental mammals) should impact development of critical brain structures in their offspring. This challenges the perception that while an embryo may draw essential nutrients from the maternal system, it still maintains virtual control over its own destiny through self-regulated expression and interactions of its own genes. However, if what I have surmised here is true – that growth and development during early embryonic life is influenced less by an individual's own genotype than by the precise rhythm of maternally-supplied THs – the primacy of foetal genes in early development is usurped. In return, however, the thyroid rhythm theory offers a testable hypothesis to address the more-critical-now-than-ever-before issue of how distinct species-specific growth and development is achieved

generation after generation, even between species with few known genetic differences between them (such as brown and polar bears, or humans and chimpanzees).

The possibility that disruptively high levels of exogenous THs, ingested by mothers as a consequence of dietary changes associated with colonization of new habitats, could not only select for particular patterns of hormonally-programmed growth of offspring but may be capable of permanently altering their THRs, introduces a "wild-card" factor into development and selection that may explain the rather sudden appearance of some evolutionary novelties that are heterochronic in nature, such those associated with bipedal morphology in early hominids. Such a mechanism - capable of by-passing the gradual accumulation of mutations in independent genes – has long been suspected to exist (e.g. see Schwartz's (1999) discussion of Goldschmidt's work).

In addition, the thyroid rhythm theory provides potential resolution of the dilemma of why, in so many cases (including instances of evolutionary novelties), a wide variety of species traits appear to have transformed simultaneously. Many such traits are often used in traditional cladistic analyses of hominids, as well as in other mammalian lineages, as if they were entirely independent characters, a practice that violates the requirement that only evolutionarily and developmentally independent characters be used for comparison (see review by Hlusko 2004). Hormonally-linked traits can be just as interdependent as genetically-linked traits and it should be apparent from evidence presented here that hormonally-linked traits need to be identified before evolutionary trends can be assessed by cladistic methods.

Regarding the long-standing debate on whether rapid evolutionary change is even possible, let alone common, detailed evidence from deliberate and experimental

domestication in silver foxes and salmonids, as well as data derived from analysis of fluctuationg adaptation in Darwin's finches over a thirty-year period (Sections 1.4, 3.23, 3.33), gives us the fastest concrete time frame yet for "rapid" speciation and adaptation events. For some taxa, significant changes can be seen within a single generation, while in others it may take up to 20 generations. This definition of "fast" stands in marked contrast to previous estimates of thousands of years for rapid speciation events (e.g. Mayr 1988) and provides a whole new perspective on the ability of populations to respond quickly to changing environmental conditions.

Lastly, the possibility that the thyroid rhythm theory might apply to organisms other than vertebrates, at least in general terms, is intriguing. The molecular character of TH is not only stable across all phyla but THs appear to be essential to mitochondrial and nuclear function in virtually all taxa. The wide-spread presence of THs in both plants and invertebrate animals, as well as the revelation that regulatory hormones in these organisms bear a striking molecular similarity to THs (Section 3.34), all suggest that THs, or molecules analogous to them, regulate development in a very wide variety of species. Since we have firm evidence that rhythmic hormonal secretion occurs in plants and invertebrates as well as vertebrates, this suggests the possibility that my theory, taken in its broadest sense, might drive adaptation and speciation in all multi-cellular organisms.

The thyroid rhythm theory is based on the premise that a simple biological mechanism exists that allows species (populations of individuals) to adapt and transform over evolutionary time in response to changing environmental conditions; this mechanism merely expands in scope the existing system individual animals are known to use in adapting to daily and seasonal changes in their lifetime. If my assertion is upheld - that

individual variants of THR phenotypes effectively control a suite of inter-connected physiological, morphological and behavioural characteristics – then the concept describes an avenue for natural selection to tap into an extremely critical reservoir of variation within a population that is not exclusively genetic in nature. Because THs influence an enormous range of cellular functions in a dose- and time-dependent manner (via genomic and non-genomic actions), selection for particular THRs should produce well coordinated and rapid changes in all biological systems simultaneously, a factor that surely must be important in creating viable alternative morphologies in response to changing environmental conditions over evolutionary time.

Using protodomestication as a model for speciation of all taxa (including humans), as I have done here, highlights the critical role played by thyroid hormone metabolism in vertebrate development and adaptation. Decoupling the speciation process of protodomestication from subsequent cultural processes provides a broad theoretical framework with enormous explanatory potential for evolutionary biology. Such a paradigm has the power to demonstrate how the developmental changes associated with heterochrony could actually drive evolution in the mode described so eloquently by the concept of punctuated equilibrium, not only in vertebrates but perhaps in invertebrates and plants as well.

REFERENCES

Aarseth, J.J., Van't Hof, T.J., and K.-A. Stokkan. 2003. Melatonin is rhythmic in newborn seals exposed to continuous light. *Journal of Comparative Physiology B* 173:37-42.

Ádám, G., Perrimon, N., and S. Noselli. 2003. The retinoic-like juvenile hormone controls the looping of left-right asymmetric organs in *Drosophila. Development* 130:2397-2406.

Adcock, C.J., Ogily-Stuart, A.L., Robinson, I.C., Lewin, J.E., Holly, J.M., Harris, D.A., Watts, A.P., Doyle, K.L., Mathews, D.R., Wilkinson, A.R., and D.B. Dunger. 1997. The use of an automated microsampling system for the characterization of growth hormone pulsatility in newborn babies. *Pediatric Research* 42(1):66-71.

Aiello, L.C., and P. Wheeler. 1995. The expensive tissue hypothesis: the brain and digestive system in human and primate evolution. *Current Anthropology* 36: 199-221.

Alberch, P. 1985a. Problems with the interpretation of developmental sequences. *Systematic Zoology* 34:46-58.

Alberch, P. 1985b. Developmental constraints: why St. Bernards often have an extra digit and poodles never do. *American Naturalist* 126:430-433.

Alberch, P. 1991. From genes to phenotype: dynamical systems evolvability. *Genetica* 84:5-11.

Alderson, L. 1978. *The Chance to Survive: Rare Breeds in a Changing World.* Cameron and Tayleur Books Ltd., London.

Anderson, G.W., Mariash, C.N. and J.H. Oppneheimer. 2000. Molecular actions of thyroid hormone. In L.D. Braverman and R.D. Utiger (eds.), *Werner and Ingbar's The Thyroid, Eighth Edition*. pp. 174-195. Lippincott, Philadelphia.

Arnason, U., Bodin, K., Gullberg, A., Ledje, C. and S. Mouchaty. 1995. A molecular view of pinniped relationships with particular emphasis on the true seals. *Journal of Molecular Evolution* 40:78-85.

Arnason, U., Gullberg, A., and B. Widegren. 1993. Cetacean mitochondrial DNA control region: sequences of all extant baleen whale and two sperm whale species. *Molecular Biology and Evolution* 10:960-970.

Arnason, U. and A. Gullberg. 1994. Relationship of baleen whales established by cytochrome b gene sequence comparison. *Nature* 367:726-728.

Arnold, M.L. 1997. *Natural Hybridization and Evolution*. Oxford University Press, Oxford.

Arnould, A.P., Xu, J., Grisham, W., Chen, X., Kim, Y.-H. and Y. Itoh. 2004. Sex chromosomes and brain sexual differences. *Endocrinology* 145(3):1057-1062.

Arons, C.D. and W.J. Shoemaker. 1992. The distribution of catecholamines and beta-endorphin in the brains of three behaviorally distinct breeds of dogs and their F1 hybrids. *Brain Research* 594:31-39.

Asdell, S.A. 1964. *Patterns of Mammalian Reproduction*. Cornell University Press, Ithaca.

Avise, J.C. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. *Evolution* 43:1191-1208.

Bandyopadhyay, A., Roy, P., and S. Bhattachacharya. 1996. Thyroid hormone induces the synthesis of a putative protein in the rat granulose cell which stimulates progesterone release. *Journal of Endocrinology* 150(2):309-318.

Banfield, A.W.F. 1974. *Mammals of Canada*. National Museum of Canada, University of Toronto Press, Toronto.

Barres, B.A., Lazar, M.A. and M.C. Raff. 1994. A novel role for thyroid hormone, glucocorticoids and retinoic acid in timing oligodendrocyte development. *Development* 120:1097-1108.

Bassett, J.H.D. and G.R. Williams. 2003. The molecular actions of thyroid hormone in bone. *Trends in Endocrinology and Metabolism* 14(8):356-364.

Bede, J.C., Teal, P.E.A., Goodman, W.G., and S.S. Tobe. 2001. Biosynthetic pathway of insect juvenile hormone III in cell suspension cultures of the sedge, *Cyperus iria*. *Plant Physiology* 127:584-593.

Behnke, R.J. 1992. *Native Trout of Western North America*. American Fisheries Society Monograph 6, Bethesda.

Belyaev, D.K. 1979. Destabilizing selection as a factor in domestication. *Journal of Heredity* 70:301-308.

Belyaev, D.K. 1984. Foxes. In I.L. Mason (ed.), *Evolution of Domesticated Animals*, pp. 211-214. Longman Co., London.

Belyaev, D.K. and L.N. Trut. 1975. Some genetic and endocrine effects of selection for domestication in silver foxes. In M.W. Fox (ed.), *The Wild Canids: Their*

Systematics, Behavioral Ecology and Evolution, pp. 416-426. Behavioral Science Series, Van Nostrand Reinhold Co., New York.

Belyaev, D.K., Ruvinsky, A.O. and L.N. Trut. 1981. Inherited activation-inactivation of the star gene in foxes:its bearing on the problem of domestication. *Journal of Heredity* 72:267-274.

Belyaev, D.K., Trut, L.N., and A.O Ruvinsky. 1975. Genetics of the W locus in foxes and expression of its lethal effects. *Journal of Heredity* 66:331-338.

Benecke, N. 1987. Studies on early dog remains from northern Europe. *Journal of Archaeological Science* 14:31-45.

Bennett, D.C. 1991. Color genes, oncogenes and melanocyte differentiation. *Journal of Cell Science* 98:135-139.

Berg, O., Gorbman, A., and H. Kobayashi. 1959. The thyroid hormones in invertebrates and lower vertebrates. In A. Gorbman (ed.), *Comparative Endocrinology:Proceedings of the Columbia University Symposium on Comparative Endocrinology, 1958.* pp. 302-319. J. Wiley and Sons, Inc., New York.

Berge, C. 2002. Peramorphic processes in the evolution of the hominid pelvis and femur. In N. Minugh-Purvis and K. McNamara (eds.) *Human Evolution Through Developmental Change*, pp. 381-404. Johns Hopkins University Press, Baltimore.

Bernal, J. 2002. Action of thyroid hormone in brain. *Journal of Endocrinological Investigations.* 25(3):268.288.

Bernatchez, L., Glemet, H., Wilson, C.C. and R.G. Danzmann 1995. Introgression and fixation of Arctic char (*Salvelinus alpinus*) mitochondrial genome in an allopatric population of brook trout (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences* 52:179-185.

Berry, R.J. 1984. House mouse. In I.L. Mason (ed.), *Evolution of Domesticated Animals.* pp. 273-283. Longman Co., London.

Berta, A., and J.L. Sumich. 1999. *Marine Mammals: Evolutionary Biology.* Academic Press, San Diego.

Bhattachacharya, S., Guin, S., Bandyopadhyay, A., Jana, N.R. and S. Halder. 1996. Thyroid hormone induces the generation of a novel putative protein in piscine ovarian follicle that stimulates the conversion of pregnenolone to progesterone. *European Journal of Endocrinology* 134(1): 128-135.

Bitman, J., Kahl, S., Wood, D.L., and A.M. Lefcourt. 1994. Circadian and ultradian rhythms of plasma thyroid hormone concentrations in lactating dairy cows. *American Journal of Regulatory, Integrative and Comparative Physiology* 266:1797-1803.

Blache, D., Blackberry, M.A., Van Cleeff, J., and G.B. Martin. 2001. Plasma thyroid hormones and growth hormone in embryonic and growing emus (*Dromaius novaehollandiae*). *Reproduction, Fertility and Development.* 13(2-3):125-132.

Boissy, A. 1995. Fear and fearfulness in animals. *Quarterly Revue of Biology* 70: 165-191.

Boitani, L.., Francisci, F., Ciucci, P. and G. Andreoli. 1995. Population biology and ecology of feral dogs in central Italy. In J. Serpell (ed.), *The Domestic Dog: Its Evolution, Behaviour and Interactions with People,* pp. 217-244. Cambridge University Press, Cambridge.

Bőkőnyi, S. 1984. Horse. In I.L. Mason (ed.), *Evolution of Domesticated Animals,* pp. 162-173. Longman Co., London.

Bowling, A.T. 1994. Dominant inheritance of overo spotting in paint horses. *Journal of Heredity* 85:222-224.

Budiansky, S. 1992. *The Covenant of the Wild: Why Animals Chose Domestication.* Weidenfeld and Nicolson, London.

Bultman, S.J., Michaud, E.J. and R.P. Woyckik 1992. Molecular characterization of the mouse *agouti* locus. *Cell* 71:1195-1204.

Burchill, S.A., Ito, S. and A.J. Thody. 1993. Effects of melanocyte-stimulating hormone on tyrosinase expression and melanin synthesis in hair follicular melanocytes of the mouse. *Journal of Endocrinology* 137:189-195.

Burrow, G.N. 1997. Editorial: mothers are important! *Endocrinology* 138:3-4.

Buyse, J., Tixier-Boichard, M, Berghman, L.R., Huybrechts, L.M. and E. Decuypere. 1990. Growth hormone secretory characteristics of sex-linked dwarf and normal-sized chickens reared on a control or on a 3, 3',5-triiodo-thyronine-supplemented diet. *General and Comparative Endocrinology* 93:406-410.

Bradley, D.G., Loftus, R.T., Cunningham, P. and D.E. MacHugh. 1998. Genetics and domestic cattle origins. *Evolutionary Anthropology* 6:79-86.

Breen, S., Rees, S. and D. Walker. 1996. The development of diurnal rhythmicity in fetal suprachiasmatic neurons as demonstrated by fos immunohistochemistry. *Neuroscience* 74(3):917-926.

Brent, G.A., 2000 Tissue-specific actions of thyroid hormone: insights from animal models. *Revue of Endocrinology and Metabolic Disorders* 1:27-33.

Brent, G.A., Moore, D.D. and P.R. Larsen. 1991. Thyroid hormone regulation of gene expression. *Annual Revue of Physiology* 53:17-35.

Carroll, S.B. 2003. Review: Genetics and the making of *Homo sapiens. Nature* 422: 849-857.

Campos-Barros, A., Musa, A., Flechner, A., Hessenius, C., Gaio, U., Meinhold, H. and A.Baumgartner. 1997. Evidence for circadian variations of thyroid hormone concentrations and type II 5'iodothyronine deiodinase activity in the rat central nervous system. *Journal of Neurochemistry* 68(2):795-803.

Chadwick, D.J. and J.A Goode. 2000. *Mechanisms and Biological Significance of Pulsatile Hormone Secretion.* J. Wiley and Sons, Chichester.

Chan, S. and M.D. Kilby. 2000. Review: Thyroid hormone and central nervous system development. *Journal of Endocrinology* 165:1-8.

Chesemore, D.L. 1975. Ecology of the arctic fox (*Alopex lagopus*) in North America: a review. In M.W. Fox (ed.), *The Wild Canids: Their Systematics, Behavioral Ecology and Evolution,* pp. 143-163. Behavioral Science Series, Van Nostrand Reinhold Co., New York.

Chino, Y., Saito, M., Yamasu, K., Suyemitsu, T, and K. Ishihara. 1994. Formation of the adult rudiment of sea urchins is influenced by thyroid hormones. *Developmental Biology* 161:1-11.

Clark, A.G., Glanowski, S., Nielsen, R., Thomas, P.D., Kejariwal, A., Todd, M.A., Tanenbaum, D.M., Civello, D., Lu, F., Murphy, B., Ferriera, S., Wang, G., Zheng, X., White, T.J., Sninsky, J.J., Adams, M.D. and M. Cargill. 2003. Inferring nonneutral evolution from human-chimp-mouse othologous gene trios. *Science* 302:1960-1963.

Clark, P.A. and A.D. Rogol. 1996. Growth hormones and sex steroid interactions at puberty. *Endocrinology and Metabolism Clinics of North America.* 25(3):665-681.

Clutton-Brock, J. 1981. *Domesticated Animals From Early Times.* British Museum (Natural History), Heinemann.

Clutton-Brock, J. 1992a. Domestication of animals. In S. Jones, R. Martin, and D. Pilbeam (eds.), *The Cambridge Encyclopedia of Human Evolution,* pp. 380-385. Cambridge University Press, Cambridge.

Clutton-Brock, J. 1992b. The process of domestication. *Mammal Revue* 22:79-85.

Clutton-Brock, J. 1995. Origins of the dog: domestication and early history. In J. Serpell (ed.), *The Domestic Dog: Its Evolution, Behaviour and Interactions with People,* pp. 8-20. Cambridge University Press, Cambridge.

Cogburn, L.A. and R.M. Freeman. 1987. Response surface of daily thyroid hormone rhythms in young chickens exposed to constant ambient temperature. *General and Comparative Endocrinology* 68(1):113-123.

Collis, K., Roby, D.D., Craig, D.P., Ryan, B.A. and R.D. Ledgerwood. 2001. Avian predation on juvenile salmonids tagged with passive integrated transponders in the Columbia River estuary: vulnerability of different salmonid species, stocks, and rearing types. *Transactions of the American Fisheries Society* 130:385-396.

Cooper, A., Mourer-Chauvire, C., Chambers, G.K., von Haeseler, A., Wilson, A.C. and S. Paabo. 1992. Independent origins of New Zealand moas and kiwis. *Proceedings of the National Academy of Sciences USA* 89:8741-8744.

Copinschi, G., Spiegel, K., Leproult, R., and E. Van Cauter. 2000. Pathophysiology of human circadian rhythms. In D.J. Chadwick and J.A Goode. (eds.), *Mechanisms and Biological Significance of Pulsatile Hormone Secretion,* pp. 143-162. J. Wiley and Sons, Chichester.

Coppinger, R. and M. Feinstein. 1991. 'Hark! Hark! The dogs do bark...' and bark and bark. *Smithsonian* 21:119-129.

Coppinger, R. and L. Coppinger. 2001. *Dogs: A New Understanding of Canine Origins,Behavior and Evolution.*. University of Chicago Press.

Coppinger, R. and R. Schneider. 1995. Evolution of working dogs. In J. Serpell (ed.), *The Domestic Dog: Its Evolution, Behaviour and Interactions with People*, pp. 21-47. Cambridge University Press, Cambridge.

Corbet, G.B. and J. Clutton-Brock. 1984. Appendix: taxonomy and nomenclature. In I.L. Mason (ed.), *Evolution of Domesticated Animals*, pp. 434-438. Longman Co., London.

Cowling, K., Robbins, R.J., Haigh, G.R., Teed, S.K. and W.D. Dawson. 1994. Coat color genetics of *Peromyscus*: IV. Variable white, a new dominant mutation in the deer mouse. *Journal of Heredity* 85:48-52.

Crabtree, P.J. 1993. Early animal domestication in the middle East and Europe. In M.B. Schiffer (ed.), *Archaeological Method and Theory*, pp. 201-245.

University of Arizona Press, Tucson.

Crawford, R.D. 1984. Turkey. In I. L. Mason (ed.), *Evolution of Domesticated Animals*, pp. 325-334. Longman Co., London.

Crockford, S.J. 1997. *Osteometry of Makah and Coast Salish dogs.* Archaeology Press No. 22, Simon Fraser University, Burnaby.

Crockford S.J. 2000a. Dog evolution: a role for thyroid hormone physiology in domestication changes. In: Crockford, S.J. (Ed.) *Dogs Through Time: An Archaeological Perspective,* pp. 11-20.. British Archaeological Reports S889, Oxford.

Crockford, S.J. 2000b. A commentary on dog evolution: regional variation, breed development and hybridisation with wolves. In: Crockford, S.J. (Ed.) *Dogs Through Time: An Archaeological Perspective,* pp. 295-312. British Archaeological Reports S889, Oxford.

Crockford, S.J. 2002a. Animal domestication and heterochronic speciation: the role of thyroid hormone. In: Minugh-Purvis, N. and McNamara, K. (Eds.) *Human Evolution Through Developmental Change*, , pp. 122-153. Johns Hopkins University Press, Baltimore.

Crockford, S.J. 2002b. Thyroid hormone in Neandertal evolution: A natural or a pathological role? *Geographical Revue,* 92: 73-88.

Crockford, S.J. 2003. Thyroid hormone rhythms and hominid evolution: a new paradigm implicates pulsatile thyroid hormone secretion in speciation and adaptation changes. *International Journal of Comparative Biochemistry and Physiology Part A – Molecular & Integrative Physiology* 135(1):105-129.

Cronin, M.A., Renecker, L., Pierson, B.J. and J.C. Patton. 1995. Genetic variation in domestic reindeer and wild caribou in Alaska. *Animal Genetics* 26:427-434.

Crowder, L.B., Rice, J.A., Miller, T.J. and E.A. Marschall. 1992. Empirical and theoretical approaches to size-based interactions and recruitment variability in fishes. In D.L. DeAngelis and L.J.Gross (eds.), *Individual-based Models and Approaches in Ecology,* pp. 237-255. Chapman and Hall, New York.

Darwin, C. 1859. *On the Origin of Species.* 1966 facsimile, J. Murray, London.

Darwin, C. 1868. *The Variation of Animals and Plants Under Domestication*, 1998 facsimile of revised ed. 1883, Johns Hopkins University Press, Baltimore.

Davis, K.D. and M.A. Lazar. 1992. Selective antagonism of thyroid hormone action by retinoic acid. *Journal of Biological Chemistry* 267:3185-3189.

Davis, P.J., Davis, F.B. and W.D. Lawrence. 1989. Thyroid hormone regulation of membrane Ca2(+)-ATPase activity. *Endocrine Research* 15(4):651-682.

Davis, S.J.M. and F.R. Valla. 1978. Evidence for domestication of the dog 12,000 years ago in the Natufian of Israel. *Nature* 276:608-610.

Davis, S.J.M. 1987. *The Archaeology of Animals.* Yale University Press, New Haven.

Dawkins, R. 1976. *The Selfish Gene.* Oxford University Press, New York.

Dawson, A., Deeming, D.C., Dick, A.C.K., and P.J. Sharp. 1996. Plasma thyroxine concentrations in farmed ostriches in relation to age, body weight, and growth hormone. *General and Comparative Endocrinology* 103:308-315.

Dawson, A., McNaughton, F.J., Goldsmith, A.R. and A.A. Degen. 1994. Ratite-like neotony induced by neonatal thyroidectomy of European starlings, *Sturnus vulgaris. Journal of Zoology London* 232:633-639.

Dayan, T. 1994. Early domesticated dogs of the Near East. *Journal of Archaeological Science* 21:633-640.

Dayan, T. and Simberloff, D. 1994. Character displacement, sexual dimorphism, and morphological variation among British and Irish mustelids. *Ecology* 75:1063-1073.

DeAngelis, D.L. and L.J. Gross. 1992. *Individual-based Models and Approaches in Ecology.* Chapman and Hall, New York.

De Groef, B., Goris, N., Arckens, L., Kűhn, E.R. and V.M. Darras. 2003. Corticotropin- releasing hormone (CRH)-induced thyrotropin release is directly mediated through CRH receptor type 2 on thyrotropes. *Endocrinology* 144(12):5537-5544.

De Pablo, F. 1993. Introduction. In Schreibman, M.P., Scanes, C.G. and P.K.T. Pang (eds.), *The Endocrinology of Growth, Development, and Metabolism of Vertebrates,* pp. 1-11. Academic Press, New York.

deMenocal, P.B. 2004. African climate change and faunal evolution during the Pliocene-Pleistocene. *Earth and Planetary Science Letters* 6976:1-22.

Denver, R.J. 1999. Evolution of the corticotropin-releasing hormone signaling system and its role in stress-induced phenotypic plasticity. *Annals of the New York Academy of Sciences* 897:46-53.

De Vries, G.J. 2004. Sex differences in adult and developing brains: compensation,

compensation, compensation. *Endocrinology* 145(3):1063-1068.

Diamond, J. 1999. *Guns, Germs and Steel: The Fates of Human Societies.* W.W. Norton & Co., New York.

Diamond, J. 2002. Evolution, consequences and future of plant and animal domestication. *Nature* 418:700-707.

Dickhoff, W.W. 1993. Hormones, metamorphosis and smolting. In Schreibman, M.P., Scanes, C.G. and P.K.T. Pang (eds.), *The Endocrinology of Growth, Development, and Metabolism of Vertebrates,* pp. 519-540. Academic Press, New York.

Dubois-Dalcq., M. and K. Murray. 2000. Why are growth factors important in oligodendrocyte physiology? *Pathological Biology* 48:80-86.

Duckett, W.M., Manning, J.P. and P.G. Weston. 1989. Thyroid hormone periodicity in healthy adult geldings. *Equine Veterinary Journal* 21(2):123-125.

Dufty Jr., A..M., Clobert, J. and A.P. Møller. 2002. Hormones, developmental plasticity and adaptation. *Trends in Ecology and Evolution* 17:190-196.

Eales, J.G. 1997. Iodine metabolism and thyroid-related functions in organisms lacking thyroid follicles: are thyroid hormones also vitamins? *Proceedings of the Society for Experimental Biology and Medicine* 214: 302-317.

Eldridge, N. and S.J. Gould. 1972. Punctuated equilibria: an alternative to phyletic gradualism. In T.J.M. Schopf (ed.) *Models in Paleobiology,* pp. 82-115. Freeman and Cooper, San Francisco.

Eliceiri, B.P. and D.D. Brown. 1994. Quantitation of endogenous thyroid hormone receptors α and β during embryogenesis and metamorphosis in *Xenopus laevis. Journal of Biological Chemistry* 269(39):24459-24465.

Elinson, R.P. 1987. Change in developmental patterns: embryos of amphibians with large eggs. In R.A. Raff and E.C. Raff (eds.), *Development as an Evolutionary Process*, pp. 1-21. A.R. Liss, Inc., New York.

Episkopou, F., Maeda, S., Nishiguchi, S., Shimada, K., Gaitanaris, G.A., Gottesman, M.E. and E.J. Robertson. 1993. Disruption of the transthyretin gene results in mice with depressed levels of plasma retinol and thyroid hormone. *Proceedings of the National Academy of Sciences USA* 90:2375-2379.

Epstein, H. 1984. Ass, mule and onager. In I.L. Mason (ed.), *Evolution of Domesticated Animals,* pp. 174-184. Longman Co., London.

Epstein, H. and M. Bichard. 1984. Pig. In I.L. Mason (ed.), *Evolution of Domesticated Animals,* pp. 145-161. Longman Co., London.

Epstein, H. and I.L. Mason. 1984. Cattle. In I.L. Mason (ed.), *Evolution of Domesticated Animals,* pp. 7-27. Longman Co., London.

Evans, R.M. 1988. The steroid and thyroid hormone receptor superfamily. *Science* 240:889-895.

Fagan, B.M. 1994. *In the Beginning: An Introduction to Archaeology*. HarperCollins, New York.

Farnsworth, E. 2004. Hormones and shifting ecology throughout plant development. *Ecology* 85(1):5-15.

Ferguson, D.C. 1994. Update on diagnosis of canine hypothyroidism. In D.C. Ferguson (ed.), Thyroid disorders, pp. 515-539. *Veterinary Clinics of North America, Small Animal Practice* 24, W.B. Saunders Co., Philadelphia.

Finch, C.E. and M.R. Rose. 1995. Hormones and the physiological architecture of life history evolution. *Quarterly Revue of Biology* 70:1-52.

Flamant, F. and J. Samarut. 2003. Thyroid hormone receptors: lessons from knockout and knock-in mutant mice. *Trends in Endocrinology and Metabolism* 14:85-90.

Fleagle, J.G., 1999. *Primate Adaptation and Evolution, Second Edition.* Academic Press, San Diego.

Fleming, I.A., Agustsson, T., Finstad, B., Johnsson, J.I. and B.T. Björnsson. 2002. Effects of domestication on growth physiology and endocrinology of Atlantic salmon *Canadian Journal of Fisheries and Aquatic Science* 59:1323-1330.

Fleming, I.A., Einum, S., Jonsson, B., and N. Jonsson. 2003. Comment on "Rapid evolution of egg size in captive salmon" (1). *Science* 302: 59b; www.sciencemag.org/cgi/content/full/302/5642/59b.

Forhead, A.J. and A.L. Fowden. 2002. Effects of thyroid hormones on pulmonary and renal angiotensin-converting enzyme concentrations in fetal sheep near term. *Journal of Endocrinology* 173(1):143-150.

Fowden, A.L., Mapstone, J. and A..J. Forhead. 2001. Regulation of glucogenesis by thyroid hormones in fetal sheep during late gestation. *Journal of Endocrinology* 170(2):461-469.

Fowler, M. 1989. Physical examination, restraint and handling. In L.W. Johnson (ed.), Llama Medicine, pp. 21-35. *Veterinary Clinics of North America, Food Animal Practice* 5, W.B. Saunders Co., Philadelphia.

Fox, H.E., White, S.A., Kao, M.H.F. and R.D. Fernald. 1997. Stress and dominance in a social fish. *Journal of Neuroscience* 17(16):6463-6469.

Fox, M.W. 1978. *The Dog: Its Domestication and Behavior.* Garland STPM Press, New York.

Friis, L.K. 1985. *An investigation of subspecific relationships of the grey wolf, Canis lupus, in British Columbia.* M.Sc. thesis, University of Victoria.

Franklyn, J.A. 2000. Metabolic changes in thyrotoxicosis. In L.D. Braverman and R.D. Utiger (eds.), *Werner and Ingbar's The Thyroid, Eighth Edition,* pp. 667-672. Lippincott, Philadelphia.

Gagneux, P., Amess, B. Diaz, S., Moore, S., Patel, T., Dillmann, W., Parekh, R., Varki, A., 2001. Proteomic comparison of human and great ape blood plasma reveals conserved glycosylation and differences in thyroid hormone metabolism. *American Journal of Physical Anthropology* 115:99-109.

Gancedo, B., Alonso-Gomez, A.L., de Pedro, N., Delgado, M.J. and M. Alonso-Bedate. 1996. Daily changes in thyroid activity in the frog *Rana perizi:* variations with season. *Comparative Biochemistry and Physiology C.* 114:79-87.

Gancedo, B., Alonso-Gomez, A.L., de Pedro, N., Delgado, M.J. and M. Alonso-Bedate. 1997. Changes in thyroid hormone concentrations and total contents through ontogeny in three anuran species: evidence for daily cycles. *General and Comparative Endocrinology,* 107: 240-250.

Garcia-Segura, L.M. and M.M. McCarthy. 2004. Role of glia in neuroendocrine function. *Endocrinology* 145(3):1082-1086.

Gardahaut, M.F., Fontaine-Perus, J., Rouaud, T., Bandman, E. and R. Ferrand. 1992. Developmental modulation of myosin expression by thyroid hormones in avian skeletal muscle. *Development* 115:1121-1131.

Gautier, A. 1993. "What's in a name?": a short history of the Latin and other labels proposed for domestic animals. In A. Clasen, S. Payne and H.P. Uerpmann (eds.), *Skeletons in her Cupboard: Festschrift for Juliet Clutton-Brock,* pp. 91-98. Monograph 34, Oxbow Books, Oxford.

Gavlik, S., Albina, M. and J.L. Specker. 2002. Metamorphosis in summer flounder: manipulation of thyroid status to synchronize settling behavior, growth, and

development. *Aquaculture* 203:359-373.

Gavrilets, S. 2003. Models of speciation: what have we learned in 40 years? *Evolution* 57(10):2197-2215.

Geissler, E.N., Ryan, M.A., and D.E. Housman. 1988. The dominant-white spotting (W) locus of the mouse encodes the c-kit proto-oncogene. *Cell* 55:185-192.

Geist, V. 1971. *Mountain Sheep: A Study in Behavior and Evolution.* University of Chicago Press, Chicago.

Geist, V. 1986. On speciation in ice age mammals, with special reference to cervids and caprids. *Canadian Journal of Zoology* 65:1067-1084.

Geist, V. 1998. *Deer of the World: Their Evolution, Behavior, and Ecology.* Stackpole Books, Mechanicsburg,PA.

Gentry, A., Clutton-Brock, J. and C. P. Groves. 2004. The naming of wild animal species and their domestic derivatives. *Journal of Archaeological Science* 31:645-651.

Gerhart, J. and M. Kirschner. 1997. *Cells, Embryos and Evolution: Toward a Cellular and Developmental Understanding of Phenotypic Variation and Evolutionary Adaptability.* Blackwell Science, Malden.

German, R.Z. and S.A. Stewart. 2002. Sexual dimorphism and ontogeny in primates. In N. Minugh-Purvis and K. McNamara (eds.) *Human Evolution Through Developmental Change*, pp. 207-222. Johns Hopkins University Press, Baltimore.

Gibson, G. and D.S. Hogness. 1996. Effect of polymorphism in the Drosophila regulatory gene Ultrabithorax on homeotic stability. *Science* 271:200-203.

Gillespie, J.M.A., Chan, B.P.K., Roy, D., Cai, F. and D.D. Belsham. 2003. Expression of circadian rhythm genes in gonadotropin-releasing hormone-secreting GT1-7 neurons. *Endocrinology* 144(12):5285-5292.

Gittleman, J.L. 1989. *Carnivore Behavior, Ecology and Evolution.* Cornell University Press, Ithaca.

Giuffra, E., Kijas, J.M.H., Amarger, V., Carlborg, Ő., Jeon, J.-T. and L. Andersson. 2000. The origin of the domestic pig: independent domestication and subsequent introgression. *Genetics* 154:1785-1791.

Goldberg, T.L.. and H. Ruvolo. 1997. Molecular phylogenetics and historical biogeography of east African chimpanzees. *Biological Journal of the*

Linnean Society 61:301-324.

Gomez, J.M., Boujard, T., Boeuf, G., Solari, A. and P.Y. Le Bail. 1997. Individual diurnal profiles of thyroid hormones in rainbow trout (*Oncorhynchus myskiss*) in relation to cortisol, growth hormone, and growth rate. *General and Comparative Endocrinology* 107(1):74-83.

Goodnight, C.J. and M.J. Wade. 2000. The ongoing synthesis: a reply to Coyne, Barton, and Turelli. *Evolution* 54(1):317-324.

Goodwin, D., Bradshaw, J.W.S. and S.M. Wickens. 1997. Paedomorphosis affects agonistic visual signals of domestic dogs. *Animal Behavior* 53:297-304.

Gould, S.J. 1977. *Ontogeny and Phylogeny*. Harvard University Press, Cambridge.

Gould, S.J. 1996. *Full House: The Spread of Excellence from Plato to Darwin*. Harmony Books, New York.

Gould, S.J. 2002. *The Structure of Evolutionary Theory*. Belknap Press, Harvard.

Gould, S.J. and N. Eldridge. 1977. Punctuated equilibria: the tempo and mode of evolution reconsidered. *Paleobiology* 3:115-151.

Gould, S.J. and N. Eldridge. 1993. Punctuated equilibrium comes of age. *Nature*, 366:223-227.

Grant, P.R., and B. R. Grant. 2002 Unpredictable evolution in a 30-year study of Darwin's finches. *Science* 296:707-711.

Grumbach, M.M. 2000. Estrogen, bone, growth and sex: a sea change in conventional wisdom. *Journal of Pediatric Endocrinology and Metabolism* 13 (suppl. 6):1439-1455.

Grau, E.G., Nishioka, R.S., Specker, J.L., and H.A. Bern. 1985. Endocrine involvement in the smoltification of salmon, with specific reference to the role of the thyroid gland. In B. Lofts and W.N. Holmes (eds.), *Current Trends in Comparative Endocrinology,* pp. 491-493. Hong Kong University Press, Hong Kong.

Greenspan, S.L., Klibanski, A., Shoenfeld, D. and E.C. Ridgway. 1986. Pulsatile secretion of thyrotropin in man. *Clinical Endocrinology and Metabolism* 63(3):661-668.

Greenspan, S.L., Klibanski, A., Rowe, J.W. and D. Elahi. 1991. Age-related alterations in pulsatile secretion of TSH: role of dopaminergic regulation. *American Journal of Physiology* 260(3 Pt. 1):486-491.

Gross, M. R. 1998. One species with two biologies: Atlantic salmon (*Salmo salar*) in the wild and in aquaculture. *Canadian Journal of Fisheries and Aquatic Science* 55 (suppl.):131-144.

Guerrant Jr., E.O. 1988. Heterochrony in plants: the intersection of evolution, ecology and ontogeny. In M.L. McKinney (ed.), *Heterochrony in Evolution: A Multidisciplinary Approach,* pp. 111-133. Plenum Press, New York.

Gunaratnam, P. 1986. The effects of thyroxine on hair growth in the dog. *Journal of Small Animal Practice* 27:17-29.

Gupta, B.B.P., and Y. Premabati. 2002. Differential effects of melatonin on plasma levels of thyroxine and triiodothyronine levels in the air-breathing fish, *Clarias gariepinus*, during breeding and quiescent periods. *General and Comparative Endocrinology* 129:146-151.

Gustafsson, A. 1994. Regulation of sexual dimorphism in rat liver. In R.V. Short and E. Balaban (eds.), *The Differences Between the Sexes*, pp. 231-241. Cambridge University Press, Cambridge.

Hadley, M.E. 1984. *Endocrinology, First Edition.* Prentice-Hall, Inc., Englewood Cliffs.

Hadley, M.E. 2000. *Endocrinology, Fifth Edition.* Prentice-Hall, Inc., Englewood Cliffs.

Hafner, J.C. and M.S. Hafner 1988. Heterochrony in rodents. In M.L. McKinney (ed.), *Heterochrony in Evolution: A Multidisciplinary Approach,* pp. 217-235. Plenum Press, New York.

Haisenleder, D.J., Ortolano, G.A., Dalkin, A.C., Yasin, M. and J.C. Marshall. 1992. Differential actions of thyrotropin (TSH)-releasing hormone pulses in the expression of prolactin and TSH subunit messenger ribonucleic acid in rat pituitary genes in vitro. *Endocrinology* 130:2917-2923.

Hall, B.K. 1992. *Evolutionary Developmental Biology.* Chapman and Hall, London.

Hall, B.K. 2003. Descent with modification: the unity underlying homology and homoplasy as seen through an analysis of development and evolution. *Biological Review* 78:409-433.

Hardy, M.P., Sottas, C.M., Ge, R., McKittrick, C.R., Tamashiro, K.L., McEwen, B.S., Haider, S.G., Markham, C.M., Blanchard, R.J., Blanchard, D.C. and R.R. Satai. 2002. Trends of reproductive hormones in male rats during psychosocial stress: role of glucocorticoid metabolism in behavioral dominance. *Biology of Reproduction* 67(6):1750-1755.

Härlid, A., Arnason, U., 1999. Analysis of mitochondrial DNA nest ratite birds

within the Neognathae: supporting a neotenous origin of ratite morphological characters. *Proceedings of the Royal Society of London B*. 266:305-309.

Harris, D.R. 1996. The origins and spread of agriculture and pastoralism in Eurasia: an overview. In D.R. Harris (ed.), *The Origins and Spread of Agriculture and Pastoralism in Eurasia.* Smithsonian Institution Press, Washington, D.C.

Harris, G.W. 1959. Neuroendocrine control of TSH regulation. In A. Gorbman (ed.), *Comparative Endocrinology: Proceedings of the Columbia University Symposium on Comparative Endocrinology 1958.* pp. 202-222. J. Wiley and Sons, Inc., New York.

Harvey, S. 1990. Thyrotropin-releasing hormone: a growth hormone-releasing factor. *Journal of Endocrinology* **125**:345-358.

Hawes, R.O. 1984. Pigeons. In I.L. Mason (ed.), *Evolution of Domesticated Animals,* pp. 351-356. Longman Co., London.

Hayes, T.B. 1997. Hormonal mechanisms as potential constraints on evolution: examples from the *Anura*. *American Zoologist* 37:482-490.

Hayes, J.P. and S.H. Jenkins. 1997. Individual variation in mammals. *Journal of Mammalogy* 78:274-293.

Heath, D. D., Heath, J. W., Bryden, C. A., Johnson, R. M., and C. W. Fox. 2003. Rapid evolution of egg size in captive salmon. *Science* 299:1738-1740.

Heath, R.B. 1989. Llama anesthetic programs. In L.W. Johnson (ed.), Llama Medicine, pp. 71-80. *Veterinary Clinics of North America: Food Animal Practice* 5. W.B. Saunders Co., Philadelphia.

Hearing, V.J. 1993. Invited editorial: Unraveling the melanocyte. *American Journal of Human Genetics* 52:1-7.

Hedrick, P. W. and J.F. McDonald. 1980. Regulatory gene adaptation: an evolutionary model. *Heredity* 45:83-97.

Hemmer, H. 1990. *Domestication: The Decline of Environmental Appreciation.* Cambridge University Press, Cambridge.

Henshaw, R.E., Lockwood, R., Shideler, R. and R.O. Stephenson. 1979. Experimental release of captive wolves. In E. Klinghammer (ed.), *The Behavior and Ecology of Wolves*, pp 319-345. Garland STPM Press, New York.

Hercbergs, A. A., 1999. Spontaneous remission of cancer: a thyroid hormone-dependent phenomenon? *Anticancer Research* 19:4839-4844.

Hercbergs, A. A., Suh, J. H., Lee, S., Cohen, B. H., Stevens, G. H., Reddy, S. K., Peereboom, D. M., Elson, P. J., Gupta, M. K., and G. H. Barnett. 2002. Propylthiouracil-induced chemical hypothyroidism with high-dose tamoxifen prolongs survival with increased reponse rate in recurrent high grade glioma. [Abstract] *Proceedings of the American Association for Cancer Research* 43: 490-491.

Hercbergs, A. A., Goyal, L. K., Suh, J. H., Lee, S., Reddy, C. A., Cohen, B. H., Stevens, G. H., Reddy, S. K., Peereboom, D. M., Elson, P. J., Gupta, M. K., and G. H. Barnett. 2003. Propylthiouracil-induced chemical hypothyroidism with high-dose tamoxifen prolongs survival in recurrent high grade glioma: a phase I/II study. *Anticancer Research* 23:617-626.

Heyning, J.E. 1997. Sperm whale phylogeny revisited: analysis of the morphological evidence. *Marine Mammal Science* 13:596-613.

Hiendleder, S., Kaupe, B., Wassmuth, R. and A. Janke. 2002. Molecular analysis of wild and domestic sheep questions current nomenclature and provides evidence for domestication from two different subspecies. *Proceedings of the Royal Society of London B* 269:893-904

Hlusko, L. J. 2004. Integrating the genotype and phenotype in hominid paleontology. *Proceedings of the National Academy of Sciences USA* 101(9):2653-2657.

Hoffmann, A.A. and P.A. Parsons. 1991. *Evolutionary Genetics and Environmental Stress*. Oxford University Press, Oxford.

Holliday, T.W. 2003. Species concepts, reticulation, and human evolution. *Current Anthropology* 44(5):653-673.

Hosoda, K., Hammer, R.E., Richardson, J.A., Baynash, A.G., Cheung, J.C., Giaid, A. and M. Yanagisawa. 1994. Targeted and natural (piebald lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. *Cell* 79:1267-1276.

Hoss, M., Dilling, A., Currant, A. and S. Paabo. 1996. Molecular phylogeny of the extinct ground sloth *Mylodon darwinii*. *Proceedings of the National Academy of Sciences USA* 93:181-185.

Huang, H., Cai, L., Remo, B.F. and D.D. Brown. 2001. Timing of metamorphosis and the onset of the negative feedback loop between the thyroid gland and the pituitary is controlled by type II iodothyronine deiodinase in *Xenopus laevis*. *Proceedings of the National Academy of Sciences USA* 98(13):7348-7353.

Iljin, N.A. 1941. Wolf-dog genetics. *Journal of Genetics* 42:359-414.

Iltis, H.H. 1983. From teosinte to maize: the catastrophic sexual transmutation. *Science* 222:886-894.

Isaac, E. 1970. *Geography of Domestication.* Foundations of Cultural Geography Series, Prentice-Hall Inc., Englewood Cliffs, New Jersey.

Jablonski, N.G., and G.Chaplin. 1992. The origin of hominid bipedalism re-examined. *Archaeology in Oceania* 27(3):113-119.

Jablonski, N.G. and G. Chaplin. 2000. The evolution of human skin coloration. *Journal of Human Evolution* 39:57-106.

Jablonski, N.G. and G. Chaplin. 2003. Skin deep. *Scientific American* 13:72-79.

Jana, N.R. and S. Bhattachacharya. 1994. Binding of thyroid hormone to the goat testicular Leydig cell induces the generation of a proteinaceous factor which stimulates androgen release. *Journal of Endocrinology* 143(3):549-556.

Jansen, T., Forster, P., Levine, M. A., Oelke, H., Hurles, M., Renfrew, C., Weber, J., and K. Olek. 2002. Mitochondrial DNA and the origins of the domestic horse. *Proceedings of the National Academy of Sciences USA* 99: 10905-10910.

Janssen, R. and J. Janssen. 1989. *Egyptian Household Animals.* Shire Publications, Bucks.

Jefcoate, C. 2002. High-flux mitochondrial cholesterol trafficking, a specialized function of the adrenal cortex. *Journal of Clinical Investigation.* 110(7):881-890.

Jensen, P. 2002. Behavior of pigs. In P. Jensen (ed.), *The Ethology of Domestic Animals: An Introductory Text,* pp. 159-171. CABI Publishing, New York.

Jobling, M. 1995. *Environmental Biology of Fishes.* Chapman and Hall, London.

Johnson, L.G. 1997. Thyroxine's evolutionary roots. *Perspectives in Biology and Medicine* 40:529-535.

Johnson, L.G. 1998. Stage-dependent thyroxine effects on sea urchin development. *New Zealand Journal of Marine and Freshwater Research* 32:531-536.

Johnson, L.G. and C.M. Cartwright. 1996. Thyroxine-accelerated larval development in the crown-of-thorns starfish, *Acanthaster planci. Biological Bulletin* 190: 299-301.

Jolicoeur, P. 1959. Multivariate geographic variation in the wolf, *Canis lupus. Evolution* 13:283-299.

Jolicoeur, P. 1975. Sexual dimorphism and geographic distance as factors of skull variation in the wolf (*Canis lupus*). In M.W. Fox (ed.), *The Wild Canids: Their Systematics,Behavioural Ecology and Evolution,* pp. 54-61. Behavioral Science Series, Van Nostrand Reinhold Co., New York.

Jonsson, B., Jonsson, N. and L.P. Hansen. 1991. Differences in life history and migratory behavior between wild and hatchery reared Atlantic salmon in nature. *Aquaculture* 98:69-78.

Kadwell, M., Fernandez, M., Stanley, H., Baldi, R., Wheeler, J., Rosadio, R., and M. Bruford. 2001. Genetic analyses reveals the wild ancestors of the llama and alpaca. *Proceedings of the Royal Society London B* 268:2575-2584

Kaptein, E.M., Hays, M.T. and D.C. Ferguson. 1994. Thyroid hormone metabolism: a comparative evaluation. In D.C. Ferguson (ed.), Thyroid disorders, pp. 431-463. *Veterinary Clinics of North America, Small Animal Practice* 24, W.B. Saunders Co., Philadelphia.

Kaufman, L.S., Chapman, L.J. and C.A. Chapman. 1997. Evolution in fast forward: haplochromine fishes of the Lake Victoria region. *Endeavor* 21:23-30.

Keeler, C. 1975. Genetics of behaviour variations in colour phases of the red fox.. In M.W. Fox (ed.), *The Wild Canids: Their Systematics, Behavioural Ecology and Evolution,* pp. 399-415. Behavioral Science Series, Van Nostrand Reinhold Co., New York.

Kenyon, C. 1994. If birds can fly, why can't we? Homeotic genes and evolution. *Cell* 78:175-180.

Kemppainen, R.J. and M.E. Peterson. 1996. Domestic cats show episodic variation in plasma concentrations of adrenocorticotropin, alpha-melanocyte-stimulating hormone (alpha-MSH), cortisol and thyroxine with circadian variation in plasma alpha-MSH concentrations. *European Journal of Endocrinology* 134(5):602-609.

Kerns, J. A., Olivier, M., Lust, G., and G. S. Barsh. 2003. Exclusion of *melanocortin-1 receptor(Mc1r)* and *agouti* as candidates for dominant black in dogs. *Journal of Heredity* 94:75-79.

Kim, M., McCormick, S., Timmermans, M., and N. Sinha. 2003. The expression of PHANTASTICA determines leaflet placement in compound leaves. *Nature* 424: 438-443.

Kingdon, J. 2003. *Lowly Origin: Where, When and Why Our Ancestors First Stood Up.* Princeton University Press, Princeton.

Kingsley, R. J., Corcoran, M. L., Krider, K. L, and K. L. Kriechbaum. 2001. Thyroxine

and vitamin D in the gorgonian, *Leptogorgia virgulata.*. *Journal of Comparative Biochemistry and Physiology Part A* 129:897-907.

Kilby, M.D., Verhaeg, J., Gittoes, N., Somerset, D.A., Clark, P.M.S. and J.A. Franklyn. 1998. Circulating thyroid hormone concentrations and placental thyroid hormone receptor expression in normal human pregnancy and pregnancy complicated by intrauterine growth restriction (IUGR). *Journal of Clinical Endocrinology & Metabolism* 83(8):2964-2971.

Kirkpatrick, M. 2000. Fish found *in flagrante delicto*. *Nature* 408:298-299.

Klandorf, H. and P.J. Sharp. 1985. Feeding-induced daily rhythms in plasma thyroid hormone levels in chickens. In B. Lofts and W.N. Holmes (eds.), *Current Trends in Comparative Endocrinology*, pp. 517-519. Hong Kong University Press, Hong Kong.

Klee, H. 2003. Hormones are in the air. *Proceedings of the National Academy of Sciences USA* 100:10144-10145.

Klingenberg, C.P. and W. Ekau. 1996. A combined morphometric and phylogenetic analysis of an ecomorphological trend: pelagization in Antarctic fishes (Perciformes: Nototheniidae). *Biological Journal of the Linnean Society* 59: 143-177.

Klingenberg, C.P. and J.R. Spence. 1993. Heterochrony and allometry: lessons from the water strider genus Limnoporus. *Evolution* 47:1834-1853.

Knap, R. and M.C. Moore. 1996. Male morphs in tree lizards, *Urosaurus ornatus*, have different delayed hormonal responses to aggressive encounters. *Animal Behavior* 52:1045-1055.

Kőhrle, J. 2000. Thyroid hormone metabolism and action in the brain and pituitary. *Acta Medica Austriaca.* 27(1):1-7.

Koibuchi, N., Natsuzaki, S., Ichimura, K., Ohtake, H. and S. Yamaoka. 1996. Ontogenetic changes in the expression of cytochrome c oxidase subunit I gene in the cerebellar cortex of the perinatal hypothyroid rat. *Endocrinology* 137: 5096-5108.

Kolesnikova, L.A. 1997. Structural and functional features of the pineal gland of silver foxes: changes under the effect of domestication. In L.N. Trut and L.V. Osadchuk (eds.) *Evolutionary-genetic and Genetic-physiological Aspects of Fur Animal Domestication: A Collection of Reports*, pp. 41-54. IFASA/Scientifur, Oslo.

Koop B.F., Burbidge M., Byun A., Rink U. and S.J. Crockford. 2000. Ancient DNA evidence of a separate origin for North American indigenous dogs. In S.J.

Crockford (ed.), *Dogs Through Time: An Archaeological Perspective*, pp. 271-286. British Archaeological Reports S889, Oxford.

Korf, H-W. 1994. The pineal organ as a component of the biological clock: phylogenetic and ontogenetic considerations. In W. Pierpaoli, W. Regelson and N. Fabris (eds.) *The Aging Clock: The Pineal Gland and Other Pacemakers in the Progression of Aging and Carcinogenesis*, pp. 13-42. *Annals of the NewYork Academy of Science* 719, New York.

Kohler, M. and S. Moya-Sola, 1997. Ape-like or hominid-like? The positional behaviour of *Oreopithecus bambolii* reconsidered. *Proceedings of the National Academy of Sciences USA* 94:11747-11750.

Künzi, C. and Sachser, N. 1999. The behavioral endocrinology of domestication: A comparison between the domestic guinea pig (*Cavia aperea* f. *porcellus*) and its wild ancestor, the cavy (*Cavia aperea*). *Hormones and Behavior* 35:28-37.

Krumlauf, R. 1994. Hox genes in vertebrate development. *Cell* 78:191-201.

Kuhn, E.R., Delmotte, N.M.J. and V.M. Darras. 1983. Persistence of a circadian rhythmicity for thyroid hormones in plasma and thyroid of hibernating male *Rana ridibunda. General and Comparative Endocrinology* 50:838-894.

Kuhn, T.S., 1970. *The Structure of Scientific Revolutions, Second Edition.* University of Chicago Press, Chicago.

Kurtén, B. 1968. *Pleistocene Mammals of Europe*. Weidenfeld and Nicolson, London.

Kurtén, B. 1988. *On Evolution and Fossil Mammals*. Columbia University Press, New York.

Kurtén, B. and E. Anderson. 1980. *Pleistocene Mammals of North America*. Columbia University Press, New York.

Lamont, M.M., Vida, J.T., Hawey, J.T., Jeffries, S., Brown, R., Huber, H.H., DeLong, R. and W.K. Wayne. 1996. Genetic substructure of the Pacific harbour seal (*Phoca vitulina richardsoni)* off Washington, Oregon and California. *Marine Mammal Science* 12:402-413.

Lamoreux, M.L. and E.S. Russell. 1979. Developmental interaction in the pigmentary system of mice. *Journal of Heredity* 70:31-36.

Langridge, J. 1991. *Molecular Genetics and Comparative Evolution*. J. Wiley and Sons Inc., New York.

Lanier, J. L., Grandin, T., Green, R., Avery, D., and McGee, K. 2001. A note on

on hair whorl position and cattle temperament in the auction ring. *Applied Animal Behaviour Science* 73:93-101.

Lanza, R.P., Cibelli, J.B., Faber, D., Sweeney, R.W., Henderson, B., Nevala, W., West, M.D. and P.J. Wettstein. 2001. Cloned cattle can be healthy and normal. *Science* 294:1893-1894.

Lapseritis, J.M. and V. Hayssen. 2001. Thyroxine levels in agouti and non-agouti deer mice (Peromyscus maniculatus). *Comparative Biochemistry and Physiology A* 130:295-299.

Lavado-Autric, R., Ausó, E., Garcia-Velasco, J.V., Carmen del Arufe, M., Escobar del Rey, F., Berbel, P. and G. Morreale de Escobar. 2003. Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. *Journal of Clinical Investigation* 111:1073-1082.

Lawrence, P.A. and G. Morata. 1994. Homeobox genes: their function in *Drosophila* segmentation and pattern formation. *Cell* 78:181-189.

Leach, H. M. 2003. Human domestication reconsidered. *Current Anthropology* 44: 349-368.

Leakey, M. and A. Walker. 2003. Early hominid fossils from Africa. *Scientific American* 13;14-19.

Lehman, N., Eisenhawer, A., Hansen, K., Mech, L.D., Peterson, R.O., Gogan, P.J. and R.K. Wayne. 1990. Introgression of coyote mitochondrial DNA into sympatric North American gray wolf populations. *Evolution* 45:104-119.

Lento, G.M., Haddon, M., Chambers, G.K. and C.S. Baker. 1997. Genetic variation in southern hemisphere fur seals (*Arctocephalus* spp.):investigation of population structure and species identity. *Journal of Heredity* 88:202-208.

Leonard, J.A., Wayne, R.K., Wheeler, J., Valadez, R., Guillén, S. and C. Vilà. 2002. Ancient DNA evidence for Old World origin of New World dogs. *Science* 298: 1613-1616.

Leonard, W. R. 2003. Food for thought. *Scientific American* 13:62-71.

Leonard, W. R. and M. L. Robertson. 1997. Rethinking the energetics of bipedality. *Current Anthropology* 38:304-309.

Levin, D.A., 2002. Hybridization and extinction. *American Scientist* 90:254-261.

Lewinson, D., Harel, Z., Shenzer, P., Silbermann, M. and Z. Hochberg. 1989. Effect of thyroid hormone and growth hormone on recovery from hypothyroidism

of epiphyseal growth plate cartilage and its adjacent bone. *Endocrinology* 124(2): 937-945.

Lieberman, D.E., 2001. Another face in our family tree. *Nature* 410:419-420.

Lien, R.J. and T.D. Siopes. 1990. The relationship of plasma thyroid hormone and prolactin concentrations to egg laying, incubation behavior, and molting by female turkeys exposed to a one-year natural daylength cycle. *General and Comparative Endocrinology* 90:205-213.

Lightman, S.L., Windle, R.J., Julian, M.D., Harbuz, M.S., Shanks, N., Wood, S.A., Kershaw, Y.M., Ingram, C.D., 2000. Significance of pulsatility in the HPA axis. In D.J. Chadwick and J.A. Goode (eds.) *Mechanisms and Biological Significance of Pulsatile Hormone Secretion*, pp. 244-260. J. Wiley and Sons, Chichester.

Little, C.C. 1958. Coat color genes in rodents and carnivores. *Quarterly Revue of Biology* 33:103-137.

Loftus, R.T., MacHugh, D.E., Bradley, D.G., Sharp, P.M. and P. Cunningham. 1994. Evidence for two independent domestications of cattle. *Proceedings of the National Academy of Sciences USA* 91:2757-2761.

Longcope, C. 2000. The male and female reproductive systems in thyrotoxicosis. In L.D. Braverman and R.D. Utiger (eds.), *Werner and Ingbar's The Thyroid, Eighth Edition.* pp. 653-6658. Lippincott, Philadelphia.

Lord, E.M. and J.P. Hill. 1987. Evidence for heterochrony in the evolution of plant form. In R.A. Raff and E.C. Raff (eds.), *Development as an Evolutionary Process,* pp. 47-70. A.R. Liss Inc., New York.

Lovejoy, C. O., Cohn, M. J. and T. D. White. 1999. Morphological analysis of the mammalian postcranium: a developmental perspective. *Proceedings of the National Academy of Sciences USA* 96:13247-13252.

Lucke, C., Hehrmann, R., von Mayersbach, K. and A. von zur Muhlen. 1977. Studies on circadian variations of plasma TSH, thyroxine and triiodothyronine in man. *Acta Endocrinologica* 86(1):81-88.

Luikart, G., Gielly, L., Excoffier, L., Vigne, J.-D., Bouvet, J., Taberiet, P. 2001. Multiple maternal origins and weak phylogeographic structure in domestic goats. *Proceedings of the National Academy of Sciences USA* 98:5927-5932.

Lynch, W. 1993. *Bears: Monarchs of the Northern Wilderness.* Douglas and McIntyre, Vancouver.

McGinnity, P., Prodőhl, P., Ferguson, A., Hynes, R., Ó Maoiléidigh, N., Baker, N., Cotter, D., O'Hea, B., Cooke, D., Rogan, G., Taggart, J. and T. Cross. 2003. Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar,* as a result of interactions with escaped farm salmon. *Proceedings of the Royal Society London B* 270(1532):2443-2450.

McKinney, M.L. 1988. *Heterochrony in Evolution: A Multidisciplinary Approach.* Plenum Press, New York.

McKinney, M.L. 1998. The juvenilzed ape myth-our "overdeveloped" brain. *BioScience* 48:109-116.

McKinney, M.L. 1999. Heterochrony: beyond words. *Paleobiology* 25:149-153.

McKinney, M.L. 2002. Brain evolution by stretching the global mitotic clock of development. In N. Minugh-Purvis and K. McNamara, K. (eds.) *Human Evolution Through Developmental Change*, pp. 173-188. Johns Hopkins University Press, Baltimore.

McKinney, M.L. and J.L. Gittleman. 1995. Ontogeny and phylogeny: tinkering with covariation in life history, morphology, and behavior. In K.J. McNamara (ed.), *Evolutionary Change and Heterochrony*, pp. 21-47. J. Wiley and Sons, Chichester.

McKinney, M.L. and K.J. McNamara. 1991. *Heterochrony – The Evolution of Ontogeny.* Plenum Press, New York.

McKinnon, J.S. and H.D. Rundle. 2002. Speciation in nature: the threespine stickleback model systems. *Trends in Ecology and Evolution* 17:480-488.

McNabb, A.F.M. and D.B. King. 1993. Thyroid hormone effects on growth, development, and metabolism. In M.P. Schreibman, C.G. Scanes and P.K.T. Pang (eds.), *The Endocrinology of Growth, Development, and Metabolism of Vertebrates,* pp. 393-417. Academic Press, New York.

McNamara, J.M. and A.I. Houston. 1996. State-dependent life histories. *Nature* 380: 215-221.

McNamara, K.J., 1995. *Evolutionary Change and Heterochrony.* J. Wiley & Sons, Chichester.

McNamara, K.J., 1997. *Shapes of Time.* Johns Hopkins University Press, Baltimore.

MacHugh, D.E. and D.G. Bradley. 2001. Livestock genetic origins: Goats buck the trend. *Proceedings of the National Academy of Sciences USA* 98:5382-5384.

Mallet, J. 1995. A species definition for the modern synthesis. *Trends in Ecology and Evolution* 10: 294-299.

Manna, P.R., Tena-Sempere, M. and I.T. Huhtaneimi. 1999. Molecular mechanisms of thyroid hormone-stimulated steroidogenesis in mouse Leydig tumor cells. *Journal of Biological Chemistry* 272(9):5909-5918.

Manzano, J., Morte, B., Scanlan, T.S. and J. Bernal. 2003. Differential effects of triiodothyronine and the thyroid hormone receptor β-specific agonist GC-1 on thyroid hormone target genes in the brain. *Endocrinology* 144(12):5480-5487.

Marchetti, M.P. and G.A. Nevitt. 2003. Effects of hatchery rearing on brain structures of rainbow trout, *Oncorhynchus mykiss. Environmental Biology of Fishes* 66:9-14.

Martin, R.D., Willner, L.A. and A. Dettling. 1994. The evolution of sexual size dimorphism in primates. In R.V. Short and E. Balaban (eds.), *The Differences Between the Sexes,* pp. 159-200. University Press, Cambridge.

Mason, I.L. 1984a. *Evolution of Domesticated Animals*, Longman Co., London.

Mason, I.L. 1984b. Goat. In I.L. Mason (ed.), *Evolution of Domesticated Animals*, pp. 85-99. Longman Co., London.

Mason, I.L. 1984c. Camels. In I.L. Mason (ed.), *Evolution of Domesticated Animals*, pp. 106-115. Longman Co., London.

Maynard Smith, J. 1983. The genetics of stasis and punctuation. *Annual Revue of Genetics* 17:11-25.

Maynard Smith, J., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B., Lande, R., Raup, D., and L. Wolpert. 1985. Developmental constraints and evolution. *Quarterly Revue Biology* 60:265-287.

Mayr, E. 1982. *The Growth of Biological Thought: Diversity, Evolution and Inheritance*. The Belknap Press of University of Harvard Press, Cambridge.

Mayr, E. 1988. *Towards a New Philosophy of Biology: Observations of an Evolutionist.* Harvard University Press, Cambridge.

Mayr, E. 1991. *One Long Argument: Charles Darwin and the Genesis of Modern Evolutionary Thought.* Harvard University Press, Cambridge, Mass.

Mayr, E. 1994. Recapitulation reinterpreted: the somatic program. *Quarterly Revue of Biology* 69:223-232.

Mayr, E. 1996. The modern evolutionary theory. *Journal of Mammalogy* 77:1-7.

Mayr, E. 2001. *What Evolution Is.* Basic Book, New York.

Mech, L.D. 1970. *The Wolf.* Natural History Press, Garden City.

Meddle, S.L. and B.K. Follett. 1997. Photoperiodically driven changes in fos expression within the basal tuberal hypothalamus and median eminence of Japanese quail. *Journal of Neuroscience* 17(22):8909-8918.

Melinda, A. 2000. The initial domestication of goats. *Science* 287:2254-2257.

Mengel, R.M. 1971. A study of dog-coyote hybrids and implications concerning hybridization in Canis. *Journal of Mammalogy* 52:316-336.

Meyer, A., Kocher, T.D., Basasibwaki, P. and A.C. Wilson. 1990. Monophyletic origin of Lake Victoria ciclid fishes suggested by mitochondrial DNA sequences. *Nature* 347:550-553.

Miller, E.H., Ponce de Leon, A. and R.L. DeLong. 1996. Violent interspecific sexual behavior by male sea lions (Otariidae):evolutionary and phylogenetic implications. *Marine Mammal Science* 12:468-476.

Milne, J.A., Loudon, A.S.I., Sibbald, A.M., Curlewis, J.D. and A.S. McNeilly. 1990. Effects of melatonin and a dopamine agonist and antagonis on seasonal changes in voluntary intake, reproductive activity and plasma concentrations of prolactin and tri-iodothyronine in red deer hinds. *Journal of Endocrinology* 125:241-249.

Minugh-Purvis, N., 2002. Heterochronic change in the neurocranium and the emergence of modern humans. In N. Minugh-Purvis and K. McNamara (eds.) *Human Evolution Through Developmental Change*, pp. 479-498. Johns Hopkins University Press, Baltimore.

Minugh-Purvis, N. and S.J. Crockford. 2002. Pulsatile hormone secretion, episodic growth patterning, heterochrony and punctuated equilibrium: A unifying model. Oral paper, American Association of Physical Anthropology, April 11, Buffalo.

Minugh-Purvis, N. and K. McNamara. 2002. *Human Evolution Through Developmental Change*, Johns Hopkins University Press, Baltimore.

Morell, V. 1997. The origin of dogs: running with the wolves. *Science* 276:1647-1648.

Morey, D.F. 1990. *Cranial allometry and the evolution of the domestic dog.* Ph.D. dissertation, University of Tennessee, Knoxville.

Morey, D.F. 1992. Size, shape and development in the evolution of the domestic dog. *Journal of Archaeological Science* 13:119-145.

Morey, D.F. 1994. The early evolution of the domestic dog. *American Scientist* (July/Aug):336-347.

Morey, D.F. and M.D. Wiant. 1992. Early Holocene domestic dog burials from the North American midwest. *Current Anthropology* 33:224-229.

Morgan, B.A. and C. Tabin. 1994. Hox genes and growth: early and late roles in limb bud morphogenesis. *Development (Suppl.)*:181-186.

Morgan, M.A., Dellovade, T.L. and D.W. Pfaff. 2000. Effect of thyroid hormone administration on estrogen-induced sex behavior in female mice. *Hormones and Behavior* 37:15-22.

Morita, S., Matsuo, K., Tsuruta, M., Leng, S., Yamashita, S., Izumi, M. and S. Nagataki. 1990. Stimulatory effects of retinoic acid and triiodothyronine in rat pituitary cells. *Journal of Endocrinology* 125:251-256.

Morreale de Escobar, G., Pastor, R., Obregon, M.J. and F. Escobar del Rey. 1985. Effects of maternal hypothyroidism on the weight and thyroid hormone content of rat embryonic tissues, before and after onset of fetal thyroid function. *Endocrinology* 117:1890-1900.

Morris, R. and D. Morris. 1966. *Men and Pandas*. McGraw-Hill, New York.

Moyle, P.B. 1969. Comparative behavior of young brook trout of domestic and wild origin. *Progressive Fish Culturist* 31:51-59.

Müller, G.B. 1990. Developmental mechanisms at the origin of morphological novelty: A side-effect hypothesis. In. M.H. Nitecki (ed.) *Evolutionary Innovations,* pp. 99-130. University of Chicago Press, Chicago.

Murakami, N., Hayafuji, C. and K. Takahashi. 1984. Thyroid hormone maintains normal circadian rhythm of blood coricosterone levels in the rat by restoring the release and synthesis of ACTH after thyroidectomy. *Acta Endocrinologia* 107(4):519-524.

Naumenko, E.V. 1973. *Central Regulation of the Pituitary-Adrenal Complex.* Studies in Soviet Science, Consultants Bureau, New York.

Noren, J.G. and J. Alm. 1983. Congenital hypothyroidism and changes in the enamel of deciduous teeth. *Acta Paediatrica Scandinavica* 72(4):485-489.

Nijhout, H.F. 1999. Control mechanisms of polyphenic development in insects. *BioScience* 49(3):181-192.

Novacek, M.J. 1992. Mammalian phylogeny: shaking the tree. *Nature* 356:121-125.

Novacek, M.J. 1993. Reflections on higher mammalian phylogenetics. *Journal of Mammalian Evolution* 1:3-30.

Nowak, R. M. 1979. *North American Quaternary Canis*. Museum of Natural History Monograph Number 6, University of Kansas, Lawrence.

Nunez, E.A., Becker, D.V., Furth, E.D., Belshaw, B.E. and J.P. Scott 1970. Breed differences and similarities in thyroid function in purebred dogs. *American Journal of Physiology* 218:1337-1341.

Nunez, J. 1985. Microtubules and brain development: the effects of thyroid hormone. *Neurochemistry International* 7:959-968.

O'Connor, T.P. 1997. Working at relationships: another look at animal domestication. *Antiquity* 71:149-156.

Ogilvy-Stuart, A.L. and S.M. Shalet. 1992. Growth hormone and puberty. *Journal of Endocrinology* 135:405-406.

Olivier, R.C.D. 1984. Asian elephant. In I.L. Mason (ed.), *Evolution of Domesticated Animals,* pp. 185-192. Longman Co., London.

Olsen, S.J. 1985. *Origins of the Domestic Dog: The Fossil Record.* University of Arizona Press, Tucson.

Olsen, S.J. 1993. Evidence of early domestication of the water buffalo in China. In A. Clasen, S. Payne and H.P. Uerpmann (eds.), *Skeletons in her cupboard: festschrift for Juliet Clutton-Brock,* pp. 151-156. Monograph 34, Oxbow Books, Oxford.

Olson, T.A. 1981. The genetic basis for piebald patterns in cattle. *Journal of Heredity* 72:113-116.

Oppenheimer, J.H. and H.L. Schwartz. 1997. Molecular basis of thyroid hormone dependent brain development. *Endocrine Review* 18:462-475.

Osadchuk, L.V. 1997. Effects of domestication on the adrenal cortisol production in silver foxes during embryonic development. In L.N. Trut and L.V. Osadchuk (eds.) *Evolutionary-genetic and Genetic-physiological Aspects of Fur Animal Domestication: A Collection of Reports.* pp. 73-81, IFASA/Scientifur, Oslo.

Owen, R.B., Crossley, R. and T.C. Johnson. 1990. Major low levels of Lake Malawi and their implications for speciation rates in cichlid fishes. *Proceedings of the Royal Society of London B* 240:519-53.

Otte, D. and J.A. Endler (eds.) 1989. *Speciation and Its Consequences.* Sinauer, Sunderland.

Park, S.K., Solomon, D., and T. Vartanian. 2001. Growth factor control of CNS myelination. *Developmental Neuroscience* 23:327-37.

Parker, S.T., 2002. Evolutionary relationships between molar eruption and cognitive development in anthropoid apes. In N. Minugh-Purvis and K. McNamara (eds.) Human Evolution Through Developmental Change, pp. 305-316. Johns Hopkins University Press, Baltimore.

Parker, S. and M. McKinney. 1999. *Origins of Intelligence: The Evolution of Cognitive Development in Monkeys, Apes, and Humans.* Johns Hopkins University Press, Baltimore.

Pavan, W.J., Mac, S., Cheng, M. and S.M. Tilghman. 1995. Quantitative trait loci that modify the severity of spotting in piebald mice. *Genome Research* 5:29-41.

Pavan, W.J. and S.M. Tilghman. 1994. Piebald lethal (sl) acts early to disrupt the development of neural crest-derived melanocytes. *Proceedings of the National Academy of Sciences USA* 91:7159-7163.

Pedraza, P., Calvo, R., Obregon, M.J., Asuncion, M., Escobar del Rey, F. and G. Morreale de Escobar. 1996. Displacement of T4 from transthyretin by the synthetic flavonoid EMD 21388 results in increased production of T3 from T4 in rat dams and fetuses. *Endocrinology* 137:4902-4914.

Pennisi, E. 2002. A shaggy dog history (editorial). *Science* 298:1540-1542.

Pickard, M.R., Sinha, A.K., Ogilvie, L. and R.P. Ekins. 1993. The influence of the maternal thyroid hormone environment during pregnancy on the ontogenesis of brain and placental ornithine decarboxylase activity in the rat. *Journal of Endocrinology* 139:205-212.

Pincus, S.M. 2000. Orderliness of hormone release. In D.J. Chadwick and J.A. Goode, (eds.), *Mechanisms and Biological Significance of Pulsatile Hormone Secretion*, pp. 82-104. J. Wiley and Sons, Chichester.

Piosik, P.A., van Groenigen, M., van Doorn, J., Baas, F. and J.J.M. de Vijlder. 1997. Effects of maternal thryoid status on thyroid hormones and growth in congenitally hypothyroid goat fetuses during the second half of gestation. *Endocrinology* 138: 5-11.

Pirinen, S. 1995. Endocrine regulation of craniofacial growth. *Acta Odontologia Scandinavica* 53(3):179-185.

Polziehn, R.O., Strobeck, C., Sheraton, J. and R. Beech. 1995. Bovine mtDNA discovered in North American bison populations. *Conservation Biology* 9:1638-1643.

Porterfield, S.P. 2000. Thyroidal dysfunction and environmental chemicals – potential impact on brain development. *Environmental Health Perspectives* 108 (Suppl. 3): 433-438.

Potts, R. 1998. Environmental hypothesis of hominin evolution. *Yearbook of Physical Anthropology* 41:93-136.

Prasolova, L.A., Trut, L.N. and E.B. Vsevolodov. 1997. Morphology of mottling hairs in domesticated silver foxes (*Vulpes vulpes*) and relation between the expression of the star and the mottling mutation. In L.N. Trut and L.V. Osadchuk (eds.) *Evolutionary-genetic and Genetic-physiological Aspects of Fur Animal Domestication: A Collection of Reports.* pp.31-40. IFASA/Scientifur, Oslo.

Prestrud, P. and K. Nilssen. 1995. Growth, size and sexual dimorphism in arctic foxes. *Journal of Mammalogy* 76:522-530.

Price, E.O. 1984. Behavioral aspects of animal domestication. *Quarterly Revue of Biology* 59:1-32.

Provine, W.B. 1986. *Sewell Wright and Evolutionary Biology.* University of Chicago Press, Chicago.

Raff, R.A. 1996. *The Shape of Life: Genes, Development, and the Evolution of Animal Form.* University of Chicago Press, Chicago.

Raff, R.A., Anstrom, J.A., Chin, J.E., Field, K.G., Ghiselin, M.T., Lane, D.J., Olsen, G.J., Pace, N.R., Parks, A.L. and E.C. Raff. 1987. Molecular and developmental correlates of macroevolution. In R.A. Raff and E.C. Raff (eds.), *Development as an Evolutionary Process*, pp. 109-138. A.R. Liss Inc., New York.

Raja, R.H., Paterson, A.J., Shin, T.H. and J.E. Kudlow. 1991. Transcriptional regulation of the human transforming growth factor-alpha gene. *Journal of Molecular Endocrinology* 5(4):514-520.

Rand-Weaver, M., Kawauchi, H., and M. Ono. 1993. Evolution of the structure of the growth hormone and prolactin family. In M.P. Schreibman, C.G. Scanes and P.K.T. Pang (eds.), *The Endocrinology of Growth, Development, and Metabolism of Vertebrates,* pp. 13-42. Academic Press, New York.

Reed, C.A. 1984. The beginnings of animal domestication. In I.L. Mason (ed.), *Evolution of Domesticated Animals*, pp. 1-6. Longman Co., London.

Reed, K.E., 1997. Early hominid evolution and ecological change through the African Plio-Pleistocene. *Journal of Human Evolution* 32:289-322.

Reilly, S.M. 1994. The ecological morphology of metamorphosis: heterochrony and the evolution of feeding mechanisms in salamanders. In P.C. Wainwright and S.M Reilly (eds.), *Ecological Morphology: Integrative Organismal Biology,* pp. 319-338. University of Chicago Press, Chicago.

Reilly, S.M., Wiley, E.O. and D.J. Meinhardt. 1997. An integrative approach to heterochrony: the distinction between interspecific and intraspecific phenomena. *Biological Journal of the Linnaean Society* 60:119-143.

Reppert, S.M. and W.J. Schwartz. 1984. Functional activity of the suprachiasmatic nuclei in the fetal primate. *Journal of Neuroscience Letters* 46(2):145-149.

Reppert, S.M. and W.J. Schwartz. 1986. Maternal suprachiasmatic nuclei are necessary for maternal coordination of the developing circadian systerm. *Journal of Neuroscience* 6:2724-2729.

Reppert, S. M. and D.R. Weaver. 2002. Coordination of circadian timing in mammals. *Nature* 418:935-941.

Rice, W.R. and E.E. Hostert. 1993. Laboratory experiments on speciation: what have we learned in 40 years? *Evolution* 47:1637-1653.

Richardson, M.K. 1995. Heterochrony and the phylotypic period. *Developmental Biology* 172:412-421.

Rindos, D. 1984. *The Origins of Agriculture: An Evolutionary Perspective.* Academic Press, Orlando.

Rivkees, S.A., Fox, C.A., Jacobson, C. D. and S. M. Reppert. 1988. Anatomic and functional development of the suprachiasmatic nuclei in the gray short-tailed opossum. *Journal of Neuroscience* 8:4269-4276.

Robbins, L.S., Nadeau, J.H., Johnson, K.R., Kelly, M.A., Roseli-Rehfuss, L., Baack, E., Mountjoy, K.G. and R.D. Cone. 1993. Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. *Cell* 72:827-834.

Robins, A.H. 1991. *Biological Perspectives on Human Pigmentation.* Cambridge University Press, Cambridge.

Robinson, I.C.F. 2000. Control of growth hormone (GH) release by GH secretagogues. In D.J. Chadwick. and J.A. Goode (eds.) *Mechanisms and Biological Significance of Pulsatile Hormone Secretion,* pp. 206-224. J. Wiley and Sons,

Chichester.

Robinson, R. 1984a. Cat. In I.L. Mason (ed.), *Evolution of Domesticated Animals*, pp. 217-224. Longman Co., London.

Robinson, R. 1984b. Syrian hamster. In I.L. Mason (ed.), *Evolution of Domesticated Animals*, pp. pp. 263-266. Longman Co., London

Robinson, R. 1984c. Norway rat. In I.L. Mason (ed.), *Evolution of Domesticated Animals*, pp. 284-290. Longman Co., London.

Rol'nik, V.V. 1970. *Bird Embryology*. Israel Program for Scientific Translations, Jerusalem.

Rook, L., 1999. *Oreopithecus* was a bipedal ape after all: evidence from the iliac cancellous architecture. *Proceedings of the National Academy of Sciences USA* 96:8795-8799.

Root-Bernstein, R. S. 1989. *Discovering: Inventing and Solving Problems at the Frontiers of Scientific Knowledge.* Harvard University Press, Cambridge.

Ross Cockrill, W. 1984. Water buffalo. In I.L. Mason (ed.), *Evolution of Domesticated Animals,* pp. 52-62. Longman Co., London.

Roy, M.S., Geffen, E., Smith, D., Ostrander, E.A. and R.K. Wayne. 1995. Patterns of differentiation and hybridization in North American wolf-like canids revealed by analysis of microsatellite loci. *Molecular Biology and Evolution* 11:553-570.

Rűber, L. and D.C. Adams. 2001. Evolutionary convergence of body shape and trophic morphology in ciclids from Lake Tanganyika. *Journal of Evolutionary Biology* 14:325-332.

Rűsch, A., Ng, L., Goodyear, R., Oliver, D., Lisoukov, I., Vennström, B., Richardson, G., Kelly, M.W. and D. Forrest. 2001. *Journal of Neuroscience* 21(24):9792-9800.

Russell-Aulet, M., Dimaraki, E.V., Jaffe, C.A., DeMott-Friberg, R. and A.L. Barkan. 2001. Aging-related growth hormone (GH) decrease is a selective hypothalamic GH-releasing hormone pulse amplitude mediated phenomenon. *Journal of Gerontology A Biological Sciences and Medical Sciences* 56(2):M124-M129.

Ryder, M.L. 1984. Sheep. In I.L. Mason (ed.), *Evolution of Domesticated Animals,* pp. 63-84. Longman Co., London.

St. Aubin, D.J., Ridgway, S.H., Wells, R.W. and H. Rhinehardt. 1996. Dolphin thyroid and adrenal hormones: circulating levels in wild and semidomesticated *Tursiops truncatus*, and influences of sex, age, and season. *Marine Mammal*

Science 12:1-13.

Sablin, M.V. and G.A. Khlopachev. 2002. The earliest ice age dogs: evidence from Eliseevichi I. *Current Anthropology* 43(5):795-799.

Satinoff, E., Li, H., Tcheng, T.K., Liu, C., McArthur, A.J., Medanic, M. and M.U. Gillette. 1993. Do the suprachiasmatic nuclei oscillate in old rats as they do in young ones? *American Journal of Physiology* 265(5 Pt 2):R1216-R1222.

Satoh, Y. and T. Sairenji. 1997. Regulation of the expression of epidermal growth factor receptor mRNA with thyroid hormone L-3,5,3'-triiodothyronine in rat hepatoma cells. *Yonaga Acta Medica* 40:133-136.

Scanlon, M.F. and A.D. Toft. 2000. Regulation of thyrotropin secretion. In L.D. Braverman and R.D. Utiger (eds.), *Werner and Ingbar's The Thyroid, Eighth Edition.* pp. 235-253. Lippincott, Philadelphia.

Scheer, F.A.J.L., Ter Horst, G.J., van der Vliet, J. and R.M. Buijs. 2001. Physiological and anatomic evidence for regulation of the heart by suprachiasmatic nucleus in rats. *American Journal of Physiology Heart and Circulatory Physiology* 280: H1391-H1399.

Schew, W.A., McNabb, F.M.A. and C.G. Scanes. 1996. Comparison of the ontogenesis of thyroid hormones, growth hormone, and insulin-like growth factor-I in ad Libitum and food-restricted (altricial) European starlings and (precocial) Japanese quail. *General and Comparative Endocrinology* 101:304-316.

Schilthuis, J.G., Gann, A.A.F. and J.P. Brockes. 1995. Chimeric retinoic acid/thyroid hormone receptors implicate RAR-à1 as mediating growth inhibition by retinoic acid. *EMBO* 12:3459-3466.

Schmutz, S.M., Berryere, T.G., Ellinwood, N.M., Kerns, J.A. and G.S. Barsh. 2003. *MC1R* studies in dogs with melanistic mask or brindle patterns. *Journal of Heredity* 94:69-73.

Schreiber, A.M., Das, B., Huang, H., Marsh-Armstrong, N. and D.D. Brown. 2001. Diverse developmental programs of *Xenopus laevis* metamorphosis are inhibited by a dominant negative thyroid hormone receptor. *Proceeding of the National Academy of Sciences USA* 98(19):10739-10744.

Schreibman, M.P., Scanes, C.G. and P.K.T. Pang. 1993. *The Endocrinology of Growth, Development, and Metabolism in Vertebrates.* Academic Press, San Diego.

Schwartz, J.H., 1999. *Sudden Origins: Fossils, Genes, and the Emergence of Species.* J. Wiley and Sons, New York.

Scott, J.P., Fuller, J.L. and J.A. King. 1959. The inheritance of annual breeding cycles in hybrid basenji-cocker spaniel dogs. *Journal of Heredity* 50:254-261.

Scribner, K. 1993. Hybrid zone dynamics are influenced by genotype-specific variation in life-history traits: experimental evidence form hybridizing *Gambusia* species. *Evolution* 47:632-646.

Searle, A.G. 1968. *Comparative Genetics of Coat Colour in Mammals.* Logos Press Ltd., London.

Segal, J. and S.H. Ingbar. 1989. Evidence that an increase in cytoplasmic calcium is an initiating event in certain plasma membrane-mediated responses to 3,5,3'-triiodothyronine in rat thymocytes. *Endocrinology* 124:1949-1955.

Shi, Z.H. and B.K. Barrel. 1992. Requirement of thyroid function for the expression of seasonal reproductive and related changes in the Red deer (*Cervus elaphus*) stags. *Journal of Reproduction and Fertility* 94:251-260.

Shibata, S. and R.Y. Moore. 1988. Development of a fetal circadian rhythm after disruption of the maternal circadian system. *Brain Research* 469:313-317.

Shin, D.-J. and T.F. Osborne. 2003. Thyroid hormone regulation and cholesterol metabolism are connected through sterol regulatory element-binding protein-2 (SREBP-2). *Journal of Biological Chemistry* 278(36):34114-34118.

Silva, J.E. 2000. Catecholamines and the sypathoadrenal system in thyroxicosis. In L.D. Braverman and R.D. Utiger (eds.), *Werner and Ingbar's The Thyroid, Eighth Edition.* pp. 642-651. Lippincott, Philadelphia.

Silvers, W.K. 1961. Genes and the pigment cells of mammals. *Science* 134:368-373.

Silvers, W.K. 1979. *The Coat Colors of Mice: A Model for Mammalian Gene Action and Interaction.* Springer-Verlag, New York.

Simoons, F.J. 1984. Gayal or mithan. In I.L. Mason (ed.), *Evolution of Domesticated Animals,* pp. 34-39. Longman Co., London.

Siracus, L.D., Washburn, L.L., Swing, D.A., Argeson, A.C., Jenkins, N.A. and N.G. Copeland. 1995. Hypervariable yellow (Ahvy), a new murine agouti mutation: (Ahvy) displays the largest variation in coat color phenotypes of all known agouti alleles. *Journal of Heredity* 86:121-128.

Skjenneberg, S. 1984. Reindeer. In I.L. Mason (ed.), *Evolution of Domesticated Animals,* pp. 128-137. Longman Co., London.

Slattery, J.P. and S.J. O'Brien. 1995. Molecular phylogeny of the red panda

(*Ailurus fulgens*). *Journal of Heredity* 86:413-422.

Smallridge, R.C., and P.W. Ladenson. 2001. Hypothyroidism in pregnancy: consequences to neonatal health. *Journal of Clinical Endocrinology and Metabolism* 86:2349-2353.

Smith, F.A., Betancourt, J.L. and J.H. Brown. 1995. Evolution of body size in the woodrat over the past 25,000 years of climate change. *Science* 270:2012-2014.

Smith, J.A. 1989. Noninfectious diseases, metabolic diseases, toxicities and neoplastic diseases of South American camelids. In L.W. Johnson (ed), Llama Medicine, pp. 101-143. *Veterinary Clinics of North America: Food Animal Practice* 5, W.B. Saunders Co., Philadelphia.

Song, C., Hiipakka, R.A., Kokontis, J.M. and S. Liao. 1995. Ubiquitous receptor: structures, immunocytochemical localization, and modulation of gene activation by receptors for retinoic acids and thyroid hormones. In D. Henderson, D. Philibert, A.K. Roy and G. Teutsch (eds.), *Steroid Receptors and Antihormones*, pp. 38-49. Annals of the New York Academy of Science 761, New York, NY.

Southren, A.C., Olivo, J., Gordon, G.G., Vittek, J., Briner, J. and F. Rafii. 1974. The conversion of androgens to estrogen in hyperthyroidism. *Journal of Clinical Endocrinology and Metabolism* 38:207-214.

Speake, B. K., Surai, P. F., Bortolotti, G. R. 2002. Fatty acid profiles of yolk lipids of five species of wild ducks (Anatidae) differing in dietary preferences. *Journal of Zoology London* 257:533-538.

Stedman, H.H., Kozyak, B.W., Nelson, A., Thesier, D.M., Su, L.T., Low, D.W., Bridges, C.R., Shrager, J.B., Minugh-Purvis, N. and M.A. Mitchell. 2004. Myosin gene mutation correlates with anatomical changes in the human lineage. *Nature* 428:415-418.

Steinhart, P. 1996. *The Company of Wolves.* Vintage Books, New York.

Stephanou, A. and S. Handwerger. 1995. Retinoic acid and thyroid hormone regulate placental lactogen expression in human trophoblast cells. *Endocrinology* 136:933-938.

Stockard, C.R. 1941. *The Genetic and Endocrinic Basis for Differences in Form and Behavior (as elucidated by studies of contrasted pure-line dog breeds and their hybrids)*. American Anatomical Memoirs 19, Wistar Institute, Philadelphia.

Stockigt, J.R. 2000. Serum thyrotropin and thyroid hormone measurements and assessment of thyroid hormone transport. In L.D. Braverman and R.D. Utiger (eds.), *Werner and Ingbar's The Thyroid, Eighth Edition.*. pp. 376-392.

Lippincott, Philadelphia.

Streelman, J.T. and P.D. Danley. 2003. The stages of vertebrate evolutionary radiation. *Trends in Ecology and Evolution* 18:126-131.

Stringer, C. 2002. Modern human origins: progress and prospects. *Philosophical Transactions of the Royal Society of London B* 357, 563-579.

Struzik, E. 2003. Grizzlies on ice. *Canadian Geographic* 123(6):38-48.

Sweeney, B. M. 1969. *Rhythmic Phenomena in Plants*. Academic Press, London.

Talbot, S.L. and G.F. Shields. 1996a. Phylogeography of brown bears (*Ursus arctos*) of Alaska and paraphyly within the Ursidae. *Molecular Phylogenetics and Evolution* 5:477-494.

Talbot, S.L. and G.F. Shields. 1996b. A phylogeny of the bears (Ursidae) inferred from complete sequences of three mitochondrial genes. *Molecular Phylogenetics and Evolution* 5:567-575.

Tautz, D. 1996. Selector genes, polymorphisms and evolution. *Science* 271:160-161.

Tchernov, E. 1993a. The impact of sedentism on animal exploitation in the southern Levant. In H. Buitenhuis and A.T. Clason (eds.), *Archaeozoology of the Near East: Proceedings of the First International Symposium on the Archaeozoology of South-western Asia and Adjacent Areas,* pp. 10-26. Universal Book Services, Leiden.

Tchernov, E. 1993b. From sedentism to domestication-a preliminary review for the southern Levant. In A. Clasen, S. Payne and H.P. Uerpmann (eds.), *Skeletons in Her Cupboard: Festschrift for Juliet Clutton-Brock.* pp. 189-233, Monograph 34, Oxbow Books, Oxford.

Tchernov, E. and L.K. Horwitz. 1991. Body size diminution under domestication: unconscious selection in primeval domesticates. *Journal of Anthropological Archaeology* 10:54-75.

Tchernov, E. and F.F. Valla. 1997. Two new dogs, and other Natufian dogs, from the Southern Levant. *Journal of Archaeological Science* 24:65-95.

Teichert, M. 1993. Size and utilization of the most important domesticated animals in Central Europe from the beginning of domestication until the late Middle Ages. In A. Clasen, S. Payne and H.P. Uerpmann (eds.), *Skeletons in her Cupboard: Festschrift for Juliet Clutton-Brock,* pp. 235-238. Monograph 34, Oxbow Books, Oxford.

Tegelstrom, H. 1987. Transfer of mitochondrial DNA from the northern red-backed vole (*Cleithrionomys rutilus*) to the bank vole (*C. glareolus*). *Journal of Molecular Evolution* 24:218-227.

Templeton, A.R. 1989. The meaning of species and speciation: a genetic perspective. In D. Otte and J.A. Endler (eds.), *Speciation and Its Consequences,* pp. 3-27. Sinauer, Sunderland.

Terasawa, E. 2001. Luteinizing hormone-releasing hormone (LHRH) neurons: mechanism of pulsatile LHRH release. *Vitamins and Hormones* 63:91-129.

Thewissen, J.G.M. 1998. *The Emergence of Whales: Evolutionary Patterns in the Origin of Cetacea.* Plenum Press, New York.

Thommes, R.C. and J.E. Woods. 1993. Endocrine regulation of the growth/development of warm-blooded vertebrate embryo/fetuses. In M.P. Schreibman, C.G. Scanes and P.K.T. Pang (eds.), *The Endocrinology of Growth, Development, and Metabolism of Vertebrates,* pp. 495-518. Academic Press, New York.

Thompson, Jr., D.L., Rahmanian, M.S., DePew, C.L., Burleigh, D.W., DeSouza, C.J. and D.R. Colborn. 1992. Growth hormone in mares and stallions: pulsatile secretion, response to growth hormone-releasing hormone, and effects of exercise, sexual stimulation, and pharmacological agents. *Journal of Animal Science* 70:1201-1207.

Thomson, K.S. 1996. The fall and rise of the English bulldog. *American Scientist* 84: 220-223.

Tickle, C. 1991. Retinoic acid and chick limb bud development. *Development (Suppl.1)*:113-121.

Timmer, D.C., Bakker, O. and W.M. Wiersinga. 2003. Triiodothyronine affects the alternative splicing of thyroid hormone receptor alpha mRNA. *Journal of Endocrinology* 179(2):217-225.

Tomasi, T.E. and D.A. Mitchell. 1994. Seasonal shifts in thyroid function in the cotton rat (*Sigmodon hispidus*). *Journal of Mammalogy* 75:520-528.

Tomasi, T.E., Hellgren, E.C. and T.J. Tucker. 1998. Thryoid hormone concentrations in black bears (*Ursus americanus*): hibernation and pregnancy effects. *General and Comparative Endocrinology* 109:192-199.

Trut, L.N. 1988. The variable rates of evolutionary transformations and their parallelism in terms of destabilizing selection. *Journal of Animal Breeding and Genetics* 105:81-90.

Trut, L.N. 1991. The intercranial allometry and morphological changes in silver foxes (*Vulpes vulpes* Desm.) under domestication (in Russian, English abstract). *Genetika* 27:1605-1611.

Trut, L.N. 1997. Domestication of the fox:roots and effects. In L.N. Trut and L.V. Osadchuk (eds.) *Evolutionary-genetic and Genetic-physiological Aspects of Fur Animal Domestication: A Collection of Reports*, pp. 7-14, IFASA/Scientifur, Oslo.

Trut, L.N. 1999. Early canid domestication: the farm-fox experiment. *American Scientist* 87:160-169.

Trut, L.N., Dzerzhinsky, F.Ya. and V.S. Nikolsky. 1991. A principal component analysis of changes in cranial characteristics appearing in silver foxes (*Vulpes vulpes* Desm.) under domestication (in Russian, English abstract). *Genetika,* 27:1440-1449.

Trut, L.N., E.V. Naumenko and D.K. Belyaev. 1972. Change in the pituitary-adrenal function of silver foxes during selection according to behavior. *Soviet Genetics* 8:35-40.

Trut, L.N. and L.V. Osadchuk. 1997. *Evolutionary- genetic and Genetic-physiological Aspects of Fur Animal Domestication: A Collection of Reports.* IFASA/Scientifur, Oslo.

Turner, S.E. 2003. *Behavioural aspects of maternal investment and disability in mother and infant Japanese macaques (Macaca fuscata) with congenital limb malformations*. M. A. thesis, University of Victoria

Uddin, M., Wildman, D.E., Liu, G., Xu, W., Johnson, R.M., Hof, P.R., Kapatos, G., Grossman, L.I. and M. Goodman. 2004. Sister grouping of chimpanzees and humans as revealed by genome-wide phylogenetic analysis of brain gene expression profiles. *Proceedings of the National Academy of Sciences USA* 101(9):2957-2962..

Uerpmann, H.P. 1993. Proposal for a separate nomenclature of domestic animals. In A. Clasen, S. Payne and H.P. Uerpmann (eds.), *Skeletons in her Cupboard: Festschrift for Juliet Clutton-Brock*, pp. 239-241. Monograph 34, Oxbow Books, Oxford.

Unwin, M.J. and G.J. Glova. 1997. Changes in life history parameters in a naturally spawning population of Chinook salmon (*Oncorhynchus tshawytscha*) associated with releases of hatchery reared fish. *Canadian Journal of Fisheries and Aquatic Science* 54:1235-1245.

Van den Berghe, G., Wouters, P., Weekers, F., Mohan, S., Baxter, R.C., Veldhuis, J.D., Bowers, C.Y. and R. Bouillon. 1999. Reactivation of pituitary hormone release

and metabolic improvement by infusion of growth hormone-releasing peptide and thyrotropin-releasing hormone in patients with protracted critical illness. *Journal of Clinical Endocrinology & Metabolism* 84(4):1311-1323.

Veldhuis, J.D. 2000. Nature of altered pulsatile hormone release and neuroendocrine network signaling in human ageing: clinical studies of the somatotropic, gonadotropic, corticotropic and insulin axes. In D.J. Chadwick and J.A. Goode (eds.) *Mechanisms and Biological Significance of Pulsatile Hormone Secretion*, pp. 163-189. J. Wiley and Sons, Chichester.

Veldhuis, J.D., Anderson, S.M., Shah, N., Bray, M., Vick, T., Gentili, A., Mulligan, T., Johnson, M.L., Weltman, A., Evans, W.S. and A. Iranmanesh. 2001. Neuro-physiological regulation and target-tissue impact of the pulsatile mode of growth hormone secretion in the human. *Growth Hormones and IGF Research* 11 (Suppl. A):S25-S37.

Veldhuis, J.D. and C.Y. Bowers. 2003. Sex-steroid modulation of growth hormone (GH) secretory control: three-peptide ensemble regulation under dual feedback restraint by GH and IGF-I. *Endocrine* 22(1):25-40.

Vilá, C., Savolainen, P., Maldonado, J.E., Amorim, I.R., Rice, J.E., Honeycutt, R.L., Crandall, K.A., Lundeberg, J. and R.K. Wayne. 1997. Multiple and ancient origins of the domestic dog. *Science* 276:1687-1689.

Vidal-Rioja, L., Zambelli, A. and L. Semorile. 1994. An assessment of the relationships among species of Camelidae by satellite DNA comparisons. *Herediatas* 121: 283-290.

Vincent, R. E. 1960. Some influences of domestication upon three stocks of brook trout (*Salvelinis fontinalis* Mitchill). *Transactions of the American Fisheries Society* 89:35-52.

Voss, S.R. 1995. Genetic basis of paedomorphosis in the axolotl, *Ambystoma mexicanum:* a test of the single-gene hypothesis. *Journal of Heredity* 86:441-447.

Voss, S.R. and H.B. Shaffer. 1997. Adaptive evolution via a major gene effect: paedo-morphosis in the Mexican axolotl. *Proceedings of the National Academy of Science USA* 94:14185-14189.

Ward, C.V., Leakey, M. G. and A. Walker. 2001. The earliest known *Australopithecus, A. anamensis*. *Journal of Human Evolution* 41: 255-368.

Watts, A.G., Tanimura, S. and G. Sanchez-Watts. 2004. Corticotropin-releasing hormone and arginine vasopressin gene transcription in the hypothalamic paraventricular nucleus of unstressed rats: daily rhythms and their interactions with corticosterone. *Endocrinology* 145(2):529-540.

Wayne, R.K. 1986a. Limb morphology of domestic and wild canids: the influence of development on morphological change. *Journal of Morphology* 187:301-319.

Wayne, R.K. 1986b. Developmental constraints on limb growth in domestic and some wild canids. *Journal of Zoology London A* 210:381-399.

Wayne, R.K. 1986c. Cranial morphology of domestic and wild canids: the influence of development on morphological change. *Evolution* 40:243-261.

Wayne, R.K. and S.M. Jenks. 1991. Mitochondrial DNA analysis implying extensive hybridization of the endangered red wolf *Canis rufus*. *Nature* 351:565-568.

Wayne, R.K. and J.L. Gittleman. 1995. The problematic red wolf. *Scientific American* 273:36-39.

Wayne, R.K., Meyer, A., Leyman, N., Van Valkenburgh, B., Kat, P.W., Fuller, T.K., Girman, D. and S.J. O'Brien. 1990. Large sequence divergence among mitochondrial DNA genotypes within populations of eastern black-backed jackals. *Proceedings of the National Academy of Science USA* 87(5):1772-1776.

Weiss, R.E. and S. Refetoff. 1996. Effect of thyroid hormone on growth: lessons from the syndrome of resistance to thyroid hormone. *Endocrinology and Metabolism Clinics of North America* 25(3):719-730.

West-Eberhard, M.J. 1989. Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics* 20:249-278.

West-Eberhard, M.J. 2003. *Developmental Plasticity and Evolution.* Oxford University Press, Oxford.

Wheeler, P.E. 1984. The evolution of bipedality and loss of functional body hair in humans. *Journal of Human Evolution* 13:91-98.

Wheeler, P.E. 1991. The influence of bipedalism on the energy and water budgets of early hominids. *Journal of Human Evolution* 21:117-136.

White, T., Suwa, G. and B. Asfaw. 1994. *Australopithecus ramidus,* a new species of early hominid from Aramis, Ethiopia. *Nature* 371:306-312.

Whiting, M.F., Bradler, S., and T. Maxwell. 2003. Loss and recovery of wings in stick insects. *Nature* 421:264-267.

Wichman, H.A. and C.B. Lynch. 1991. Genetic variation for seasonal adaptation in Peromyscus leucopus: nonreciprocal breakdown in a population cross. *Journal of Heredity* 82:197-204.

Wilcox, B.W. and C. Walkowicz. 1989. *The Atlas of Dog Breeds of the World.* T.F.H Publications, Neptune City.

Wilson, C.M. and F.M.A. McNabb. 1997. Maternal thyroid hormones in Japanese quail eggs and their influence on embryonic development. *General and Comparative Endocrinology* 107:153-165.

Windle, R.J., Wood, S.A., Lightman, S.L. and C.D. Ingram. 1998. The pulsatile characteristics of hypothalamo-pituitary-adrenal activity in female Lewis and Fischer 344 rats and its relationship to differential stress responses. *Endocrinology* 139(10):4044-4052.

Wintle, A.G. 1996. Archaeologically-relevant dating techniques for the next century. *Journal of Archaeological Science* 23:123-138.

Witte, O.N. 1990. Steel locus defines new multipotent growth factor. *Cell* 63:5-6.

Wood, B. 2002. Palaeoanthropology: Hominid revelations from Chad. *Nature* 418: 133-135.

Wood, C.C. and C.J. Foote. 1996. Evidence for sympatric genetic divergence of anadromous and nonanadromous morphs of sockeye salmon (*Oncorhynchus nerka*). *Evolution* 50:265-1279.

Wolf, M., Ingbar, S.H. and A.C. Moses. 1989. Thyroid hormone and growth hormone interact to regulate insulin-like growth factor-I messenger ribonucleic acid and circulating levels in the rat. *Endocrinology* 125(6):2905-2914.

Woller, M.J., Everson-Binotto, G., Nichols, E., Acheson, A., Keen, K.L., Bowers, C.Y. and E. Terasaw. 2002. Aging-related changes in release of growth hormone and luteinizing hormone in female rhesus monkeys. *Journal of Clinical Endocrinology and Metabolism* 87(11):5160-5167.

Woolf, C.M. 1995. Influence of stochastic events on the phenotype variation of common white leg markings in the Arabian horse: implications for various genetic disorders in humans. *Journal of Heredity* 86:129-135.

Wrangham, R. and N. Conklin-Brittain. 2003. Cooking as a biological trait. *Journal of Comparative Biochemistry and Physiology Part A* 136:35-46.

Wright, M. L. 2002. Melatonin, diel rhythms, and metamorphosis in anuran amphibians. *General and Comparative Endocrinology* 126:251-254.

Wright, M. L., Duffy, J. L., Guertin, C. J., Alves, C. D., Szatkowski, M. C., and R. F. Visconti. 2003. Developmental and diel changes in plasma thyroxine and plasma

and ocular melatonin in the larval and juvenile bullfrog, *Rana catesbeiana*. *General and Comparative Endocrinology* 130:120-128.

Wright, M. L., Guertin, C. J., Duffy, J. L., Szatkowski, M. C., Visconti, R. F., and C. D. Alves. 2003. Developmental and diel profiles of plasma corticosteroids in the bullfrog, *Rana catesbeiana*. *Comparative Biochemistry and Physiology Part A* 135:585-595.

Wrutniak, C., Casa, F. and G. Cabello. 2001. *Journal of Molecular Endocrinology* 26: 67-77.

Wu, R., Ma, C.-X., Lou, X.-Y. and G. Casella. 2003. Molecular dissection of allometry, ontogeny, and plasticity: a genomic view of developmental biology. *BioScience* 53(11):1041-1047.

Yaoita, Y., Shi, Y.-B. and D.D. Brown. 1990. *Xenopus laevis* α and β thyroid hormone receptors. *Proceedings of the National Academy of Sciences USA* 87:7090-7094.

Yoshimura, T., Yasuo, S., Watanabe, M., Iigo, M., Yamamura, T., Hirunagi, K. and S. Ebihara. 2003. Light-induced hormone conversion of T_4 to T_3 regulates photoperiodic response of gonads in birds. *Nature* 426:178-181.

Young, S.P. 1951. *The Clever Coyote*. Wildlife Management Institute, Washington, DC.

Young, S.P and E.A. Goldman. 1944. *The Wolves of North America*. Dover Publications Inc., New York.

Yeh, J. 2002. The effect of miniaturized body size on skeletal morphology in frogs. *Evolution* 56:628-641.

Yen, P.M. 2001. Physiological and molecular basis of thyroid hormone action. *Physiological Reviews* 81(3):1097-1142.

Yen, P.M. 2003. Molecular basis of resistance to thyroid hormone. *Trends in Endocrinology and Metabolism* 14 (7): 327-333.

Zandieh Doulabi, B., Platvoet-Ter Schiphorst, M., Kalsbeek, A., Fliers, E., Bakker, O. and W.M. Wiersinga. 2004. Diurnal variation in rat liver thyroid hormone receptor (TR)-α messenger ribonucleic acid (mRNA) is dependent on the biological clock in the suprachiasmatic nucleus, whereas diurnal variation of TRβ1 mRNA is modified by food intake. *Endocrinology* 145(3):1284-1289.

Zeuner, F.E. 1963. *A History of Domesticated Animals*. Hutchinson, London.

Zoeller, R.T. 2003. Transplacental thyroxine and fetal brain development. *Journal of Clinical Investigations* 111(7):954-957.

Appendix A: Published commentary paper,

Neandertal evolution.

Crockford, S.J. 2002. Thyroid hormone in Neandertal evolution: A natural or a pathological role? *Geographical Revue,* 92: 73-88.

COMMENTARY: THYROID HORMONE IN NEANDERTAL EVOLUTION: A NATURAL OR A PATHOLOGICAL ROLE?*

SUSAN J. CROCKFORD

Jerome Dobson recently proposed a controversial explanation for the morphological characteristics found among "classic" Pleistocene Neandertals from central Europe (1998). Chronic iodine deficiency in these hominids, he suggests, either due to low dietary iodine consumption (a pathological causality) or as a consequence of genetically impaired iodine utilization (a biological causality), may have interfered with thyroid hormone metabolism to such an extent that normal human skeletal development was compromised. He states that "the number and quality of morphological similarities between cretins and Neandertals argues for iodine as a key factor in controlling Neandertal morphology. . . . Iodine deficiency, whether biological or pathological, could explain the apparent ease with which new Cro-Magnon arrivals swept aside their Neandertal predecessors. In either case, biological or pathological, recovery from cretinism may explain the total disappearance of certain Neandertal traits in subsequent populations" (p. 14). Although I believe Dobson's explanation is untenable, the apparent similarities he describes between modern endemic cretins and classic Neandertals are intriguing.

Both Neandertal and cretin bones, Dobson points out, are particularly robust and thickened: The skull is large relative to body size, and the body trunk is disproportionately long compared with the short limbs. He illustrates the disproportionately short distal limb bones (radius/ulna, tibia/fibula) characteristic of Neandertals (Aiello and Dean 1990), a trait apparently shared with cretins.

Similar skeletal traits, however, are seen in modern Inuit and Lapp (Sami) populations and in several Pleistocene carnivores, including the dire wolf and the saber-toothed cat (Stock and Lance 1948; Stock 1956; Kurtén and Anderson 1980; Trinkaus and Shipman 1993; Wolpoff 1999). These last two species, now as inexplicably extinct as other members of the Pleistocene megafauna, are described as differing from their Holocene counterparts in skull size, limb proportions, and general robustness. A massive skull, heavy bone structure, and disproportionately shortened distal limb bones compared with those of closely related taxa are not, therefore, traits unique to Neandertals or even to the hominid lineage. I suggest that these skeletal characteristics indeed reflect thyroid hormone synthesis, but in a totally

* I thank Nicholas Rolland for his continued intellectual support regarding the human evolution component of my hypothesis and Douglas Ferguson for alerting me to the enormous differences between human and carnivore thyroid hormone utilization. Thanks also to April Nowell and Nancy Minugh-Purvis for comments on a previous draft of this commentary and to both Glenn Geelhoed and Jerome Dobson for providing copies of in-press manuscripts.

Ms. CROCKFORD is a zoologist and the principal faunal analyst for Pacific IDentifications, Inc., Victoria, British Columbia, Canada V9E 2J4.

The Geographical Review 92 (1): 73–88, January 2002

natural rather than pathological manner, because of the role that thyroid hormone plays in growth and development.

The pathological changes associated with massive endemic cretinism that Dobson correlates with Neandertal morphology are reflected in the skeleton because they are a consequence of interference in normal fetal growth. This disruption in fetal growth is caused by a chronic deficiency of the iodine required for thyroid hormone synthesis in the mother, because fetuses require thyroid hormone from maternal sources for normal development (Hadley 1984; Geelhoed 1999; Crockford 2002). Iodine deficiency that continues after birth has further developmental repercussions, because the rapid brain and skeletal growth that occurs during the early postnatal period are also dependent on relatively high levels of available thyroid hormone (Hadley 1984; Geelhoed 1999). Consequently, not only do severely afflicted endemic cretins have modified skeletons, but most individuals also suffer such pronounced mental retardation that they are forever dependent on healthy individuals for basic care (Geelhoed 1999).

This dependency on thyroid hormone for normal growth and development is characteristic of all vertebrates, not just humans (Waterman 1958; Hadley 1984). Surprisingly, although the essential nature of thyroid hormone in both fetal development and postnatal growth of vertebrates is well recognized, until now the evolutionary significance of this fact was not explored to any appreciable degree. Differences in growth and development, both within and between species, reflect normal biological variation and are not anomalies unique to pathological conditions associated with iodine deficiency or impaired iodine uptake.

Changes in the rates and timing of prenatal and postnatal development in ancestral species make possible a wide variety of size and shape differences in descendant populations, a well-known evolutionary pattern called "heterochrony." It is without question the most common mode of speciation (Gould 1977; McNamara 1995). Although identification of the precise biological trigger that implements such developmental change has proved elusive, hormonal involvement has long been suspected (Hayes 1997; Voss and Shaffer 1997; McKinney 1998).

Recently, I presented a theory and a testable hypothesis to explain the role of thyroid hormone (thyroxine) in heterochronic speciation events for all vertebrate taxa, including humans (Crockford 2000, 2002). I propose that the mechanism responsible for generating new species—reproductively isolated descendant populations with a distinctive suite of well-coordinated traits—is selection for the naturally occurring variants of thyroxine production patterns (thyroid rhythms) that exist within ancestral species. Because thyroxine is an essential central regulator that links many morphological, physiological, and behavioral traits, it is strongly implicated as the pivotal agent in evolutionary change. Here I present a summary of the concept and show its ability to explain characteristic Neandertal morphology. Surprisingly, this novel paradigm for describing how developmental changes could be achieved in evolution emerged from a critical reevaluation of our understanding of animal domestication.

Domestication and Speciation

Domestic animals have traditionally been defined as products of human innovation, with foundation stocks presumably derived from young animals deliberately removed from the wild (Clutton-Brock 1992; Morey 1992). This view is entirely unsupported by scientific evidence, however: In essence, it is one of our most enduring cultural myths. Recent studies suggest instead that the initial stage in the generation of most domestic animals was probably self-directed, involving a natural process of colonization of the new, anthropogenic environments that surrounded permanent late Pleistocene and early Holocene human settlements (Tchernov 1993). I suggest that the initial process that changed some wild animals into domestic forms was a completely natural speciation event with analogs in many other taxa (Crockford 2000, 2002).

Domestication described in this way allows the initial speciation stage (protodomestication) to be decoupled from subsequent cultural influences (classic domestication). Protodomestication can then be effectively compared with speciation in nondomestic vertebrate lineages, especially those in which colonization of new habitats, or geographical speciation, is evident (Gould 1977; Mayr 1988). In other words, it allows us to investigate precisely how evolutionary processes work, using common domestic taxa as models (cf. Darwin 1859, 1868). Domestication defined in this way involves a real scientific hypothesis: It is based on explicit biological assumptions that can be tested experimentally (Crockford 2002).

Typical domestic animals—dogs, cattle, pigs, goats, sheep—share a number of characteristics that distinguish them from their wild ancestors, some of which clearly involve shifts in the rates and timing of growth and development (heterochrony). These traits include changes in overall size, head shape, and behavior (Clutton-Brock 1992). For example, our most intensely studied domesticate—the dog—has been shown to possess a different rate of growth, especially during the early postnatal period, than does its ancestral species, the grey wolf (Wayne 1986; Morey 1992). This simple developmental change appears to be responsible for the "juvenilization" in morphological and behavioral traits evident in adult dogs, a pattern further accentuated in certain breeds (Coppinger and Schneider 1995; McKinney 1998). Other traits common to domesticates—changes in reproductive physiology (especially fecundity), white spotting or piebaldness, and basic coat color—appear to be inevitable consequences of heterochrony rather than heterochronic changes themselves. The essential regulator of all traits that undergo change during domestication, including piebaldness, is thyroid hormone (Hadley 1984; Schreibman, Scanes, and Pang 1993; Crockford 2002).

Thyroid Hormone and Heterochronic Speciation

Due to its central regulatory role, thyroid hormone is strongly implicated as the pivotal agent in both protodomestication and speciation associated with colonization of new habitats. Thyroid hormone has long been implicated in amphibian evolution, for example, where heterochronic changes are common; and the same has

recently been suggested for birds (Hayes 1997; McNabb and Wilson 1997; Voss and Shaffer 1997; Härlid and Arnason 1999). This suggests a universal role for thyroid hormone as an evolutionary control mechanism in all vertebrates (Crockford 2002).

The two forms of thyroid hormone—T^3 and T^4, which vary according to the number of iodine atoms attached—are often collectively referred to as "thyroxine." Iodine-bound thyroxine precursors are manufactured and stored within the thyroid glands until thyroid-stimulating hormone (TSH) produced by the pituitary triggers the molecules to be hydrolyzed and released as thyroxine. Although the genes that govern the incorporation of iodine into thyroxine operate within the thyroid glands themselves, the genes that control the release of thyroxine into the bloodstream are located in brain tissues. Ultimately, control over TSH release (and thus thyroxine secretion) appears to come from complex interactions between electrical signals received via the retina and other nervous system receptors, the eight or more so-called clock genes that reside in circadian oscillator cells of the suprachiasmatic nuclei of the anterior hypothalamus, and the hormone melatonin produced by the pineal gland in the brain (Reppert and Weaver 2002; Wright 2002; Crockford in press). Thyroxine secretion is distinctly pulsatile in nature, due to the intermittent stimulus for thyroxine release that the thyroid glands receive from the pituitary (Wright 2002). The precise frequency and intensity of the thyroxine pulses change according to other physiological demands (Kaptein, Hays, and Ferguson 1994).

Among other roles, thyroxine is the biochemical agent responsible for coordinating the body's adaptive response to both short-term (daily) and long-term (seasonal and yearly) changes in environmental conditions. Thyroxine by itself is directly responsible for control of metabolism, early embryonic growth, and the expression of a number of strategic genes (see Crockford 2002 for more details). In addition, thyroxine is the trigger that initiates production of other essential hormones (Figure 1). These thyroxine-induced hormones govern such vital functions as fetal and postnatal growth, brain development, reproduction, coat color, and behavior in response to stress (Hadley 1984; Schreibman, Scanes, and Pang 1993).

The pulsatile pattern of thyroxine production is its most critical characteristic. Precision in timing (frequency of pulses) and absolute amounts of thyroxine produced (amplitude of pulses) are almost certainly crucial to target genes, cells, and organ tissues. The few studies undertaken so far indicate that precise patterns of thyroxine production or thyroid rhythms (blood hormone concentrations graphed against time) are species specific (Gancedo and others 1997; Crockford 2002). Consequently, thyroxine-induced hormones produced by other glands and organs (Chadwick and Goode 2000) also show species-specific profiles (Figure 2). As growth and development are so dependent on thyroxine, species-specific thyroid rhythms must control species-specific growth patterns (although other factors may modify this control at the receiving end, rather than the production end, of hormone action). In addition, the interaction of thyroid rhythms with growth and sex hormones provides an explanation for species-specific sexual dimorphism (Short and Balaban 1994).

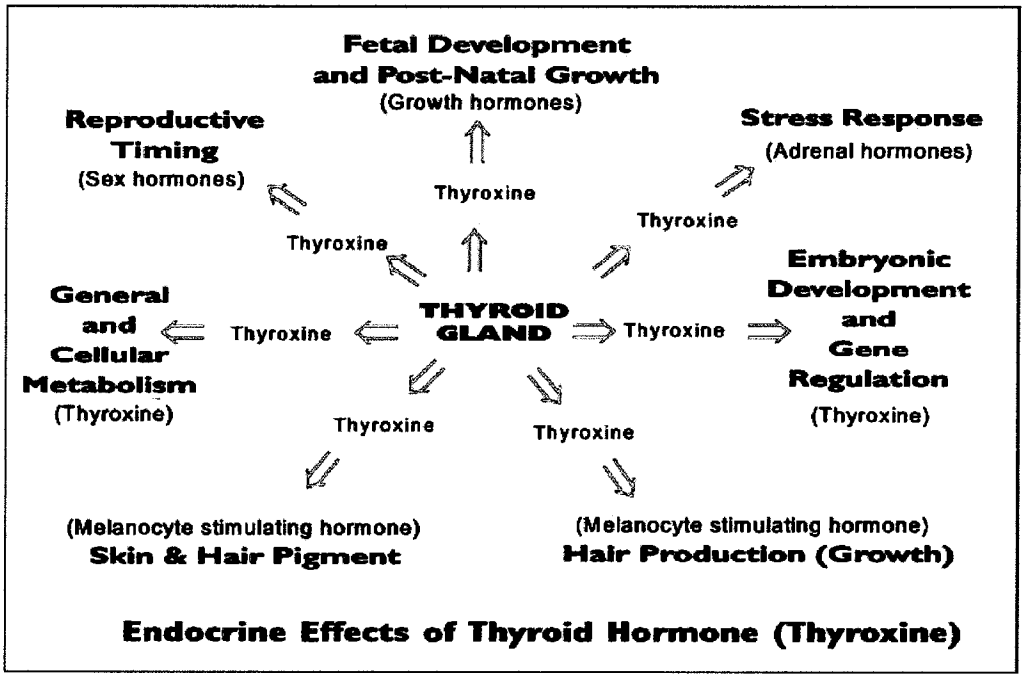

FIG. 1—A summary of endocrine effects of thyroid hormone (thyroxine). Thyroxine affects embryonic growth and metabolism directly; other systems are permissively affected. Secretion of thyroxine from the thyroid gland is initiated by thyroid-stimulating hormone produced by the pituitary gland, which is itself stimulated by the pineal/hypothalamic axis. Melanocyte-stimulating hormone initiates hair growth as well as pigment production within hair and skin follicles. *Source:* Hadley 1984.

Slight mutations in the genes that regulate the release of thyroxine produce small pattern differences within species. If precision in timing and absolute amounts of thyroxine produced are critical to different tissues at different developmental and life history stages, as I propose must be the case, then even slight variations in thyroid rhythms will result in small physiological differences among individuals or thyroid rhythm phenotypes.

These genetically controlled hormonal pattern differences constitute a particular kind of individual variation: variation for the combined output of an entire suite of genes. Because thyroxine influences so many traits and body functions, individual thyroid rhythm phenotypes must underlie much of the morphological, reproductive, and behavioral differences we recognize as individual variation within species (Gould 1977; Hayes and Jenkins 1997; Windle and others 1998; Lightman and others 2000). It is important to stress that such a suite of genes produces individual variation in seemingly unrelated traits through a complex developmental cascade, as opposed to genes that vary and operate independently. Thus selection for any one thyroxine-controlled trait also selects for all other traits linked to it by that physiological phenotype. The suite of genes that generates thyroid rhythm phenotypes is postulated as providing the essential raw material, the individual variation, for natural selection to act upon during speciation.

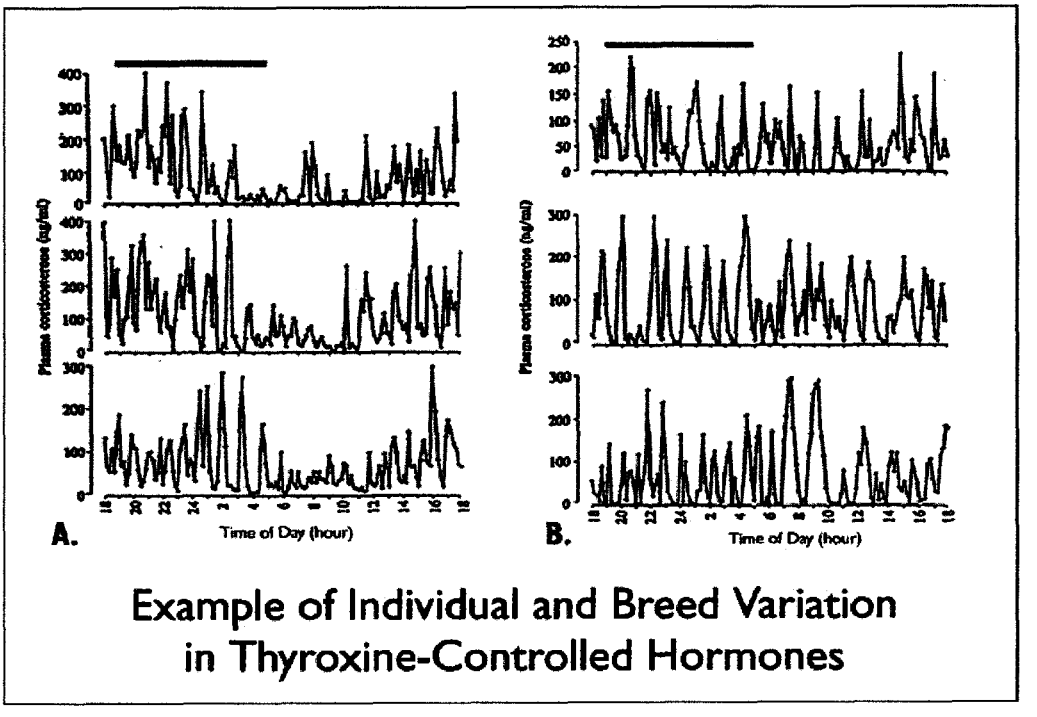

FIG. 2—Individual and breed variation in corticosterone production in two laboratory strains of rat (A, Lewis; B, Fisher). Profiles record plasma corticosterone concentrations (ng/ml) over time of day (hours); the filled bar is the ten-hour "dark" phase. Thyroxine, which ultimately controls production of adrenal hormones like corticosterone, should produce similar individual, breed-specific, and species-specific rhythms. (Reprinted with permission from Windle and others 1998, 4046).

THE THYROID RHYTHM THEORY

The theory states that the mechanism responsible for generating new species—reproductively isolated descendant populations with a distinctive suite of well-coordinated traits—is selection for the naturally occurring thyroid rhythm phenotypes that exist within ancestral species. Selection for specific thyroid rhythm phenotypes allows morphology, behavior, and reproductive strategies to change together.

The intimate role that thyroxine plays in the response of animals to stress is pivotal to this theory (Hadley 1984). Although new habitats—including anthropogenic ones, in the case of protodomestication—may offer attractive benefits, they nevertheless present stressful conditions that must be dealt with by each colonizing individual (Gould 1977). Such new habitats, therefore, are postulated as attracting physiologically stress-tolerant individuals (that is, those with particular thyroid rhythm phenotypes) preferentially over less stress-tolerant animals. I suggest that colonizing individuals with stress-tolerant physiological phenotypes thus form founder populations composed of animals that possess a nonrandom subset of the thyroid rhythm phenotypes maintained by ancestral populations (modeled in Figure 3). The variation of thyroid rhythm phenotypes existing within these small colonizing populations is much reduced.

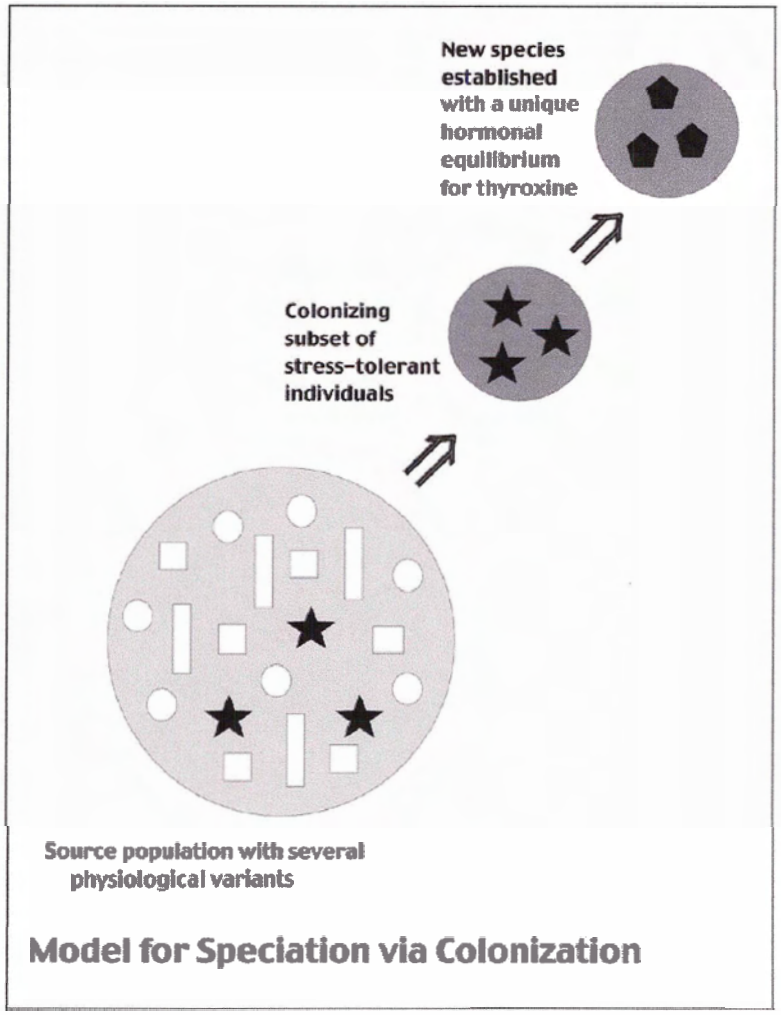

FIG. 3—A geographical model for heterochronic speciation through colonization of new habitats. In this model, shapes represent naturally occurring thyroid rhythm phenotypes (the "star" phenotype is a stress-tolerant variant; other shapes represent other thyroid rhythm phenotypes). Individuals with a relatively high tolerance of stress disperse preferentially over animals with less stress tolerance. Colonizing groups are thus composed of individuals that possess a limited range of physiological phenotypes compared with their ancestors. New species result when a new thyroid rhythm is established, one different from the ancestral pattern.

The developmental consequences of such limited variation in thyroid rhythm phenotypes isolated within small founding populations of colonizing individuals would be manifested quickly, due to the essential developmental role of thyroxine. Rapid changes in morphology, behavior, and life history are predicted to occur in descendant populations as a new hormonal rhythm for the founding group is established. Experimental domestication studies of foxes suggest that such changes

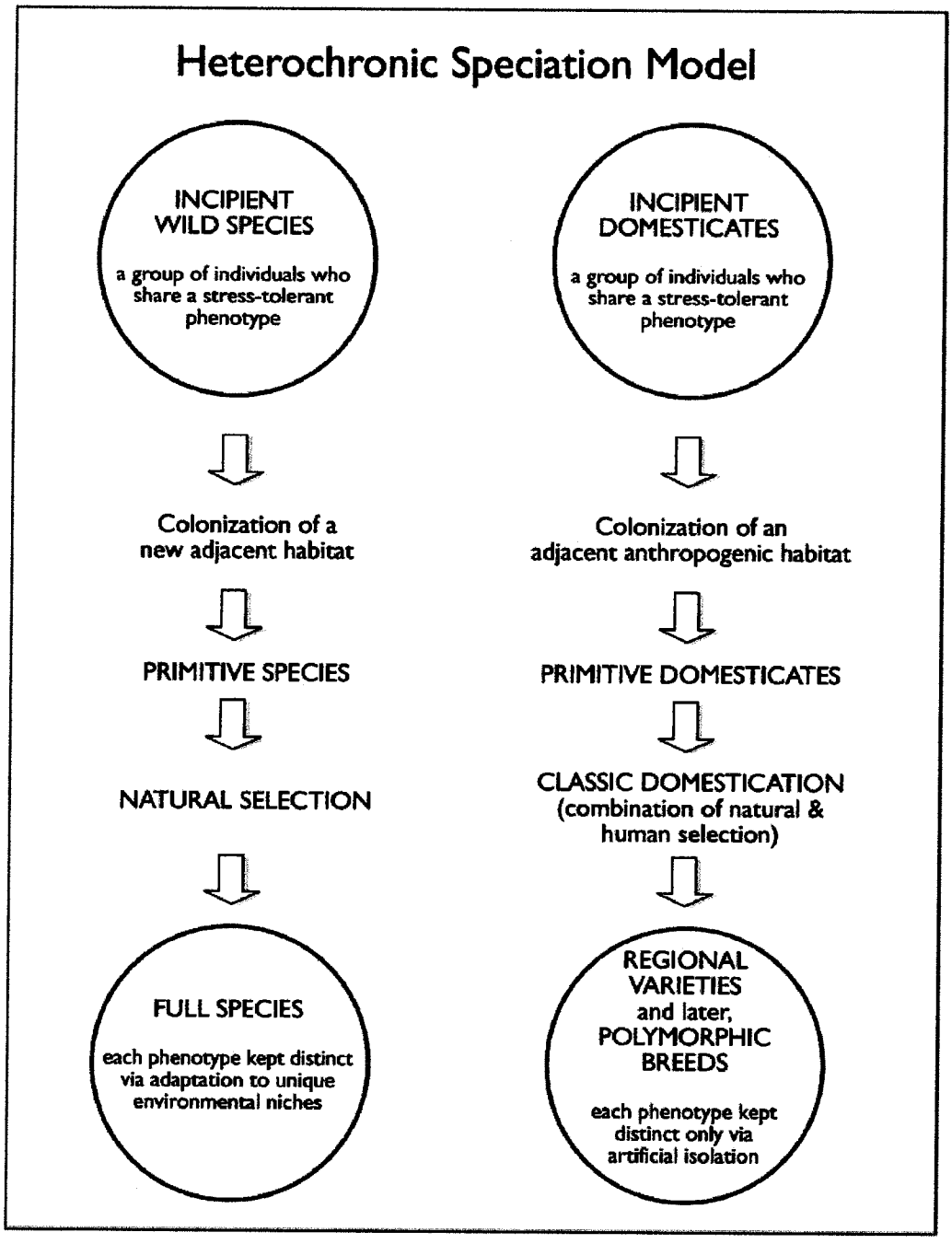

FIG. 4—A model for heterochronic speciation in vertebrates, contrasting colonization of anthropogenic and nonanthropogenic habitats.

may occur within twenty generations under artificial selection for behavioral phenotypes—almost instantaneous in evolutionary terms (Belyaev 1979; Coppinger and Schneider 1995; Trut 1999). These colonizers are morphologically, reproductively, and behaviorally distinct despite a close genetic relationship to their ancestors. Some genes from the ancestral population have actually been lost, because genes responsible for some of the original thyroid rhythm phenotypes are left behind if the individuals in which they occur do not become founders. Once such speciation change occurs it is permanent unless gene flow with the ancestral population is reestablished. The descendant populations resulting from such colonization events represent new species as soon as a unique hormonal pattern for thyroxine is achieved.

Mac Hadley defined stress as "the state resulting from events (stressors) of external or internal origin, real or imagined, that tend to affect the homeostatic state. . . . [Stress constitutes] any condition tending to elevate plasma catecholamine levels in response to exogenous or endogenous stimuli" (1984, 337). Stress can result from changes in such features of the environment as climate (light, temperature, humidity), type and quantity of predators (both real and imagined), and availability of suitable food. Existing variation in physiological tolerance of stress could inevitably lead to the nonrandom subdivision of an ancestral population in response to changes in any stress-inducing condition.

The concept explains, in essence, how evolutionary adaptation to changing environmental conditions is achieved by populations, using the same biological mechanism used by individuals. The theory applies equally to all vertebrate taxa over evolutionary time. In the case of domesticates, the stressor is the constant presence of a potential predator (humans); for other animals, including humans, the stressor is something else (Figure 4).

Explaining Neandertal Morphology

The oldest fossils of early or pre-Neandertals (ca. 300,000–350,000 years old) have been found in Spain, suggesting that Neandertals diverged out of archaic hominid populations in western Europe (Stringer and Gamble 1993; Mellars 1996; Manzi, Gracia, and Arsuaga 2000). Among the stressors that can be postulated as driving Neandertal evolution, temperature and diet figure prominently. The ancestors of Neandertals that initially colonized arctic steppe and tundra habitats in the Middle Pleistocene would have had to deal with both cold temperatures and the food limitations imposed by those conditions. Because thyroid hormone metabolism is the body's mechanism for adjusting individuals to cold, individual variation in thyroid rhythms within the ancestral population would have given some individuals a higher tolerance of the physiological stress of reduced temperatures than others. Compounding this selection pressure would have been the relatively high proportion of the diet necessarily composed of raw meat—more meat because fewer plant products were available, and raw because there is no conclusive evidence that hominids were using fire for cooking at the time (Fleagle 1999; Arsuaga 2000).

Although the bones of butchered animals found associated with Neandertals suggest a diet dominated by red meat (Mellars 1996), recent mineral analysis of Neandertal remains provides strong evidence that their diet was almost exclusively carnivorous (Balter and others 2001). A diet with such a high proportion of meat must have been a reasonably good source of iodine and of exogenous (dietary) thyroxine: Thyroxine is present in the flesh, blood, organ tissue, egg yolks, and thyroid glands of all vertebrate species (Hadley 1984; McNabb and Wilson 1997); and where there is thyroxine, there must also be iodine. Unique among hormones, thyroxine can be easily absorbed through the digestive tract (Romer 1970; Hadley 1984), and ingested thyroxine of prey origin is indistinguishable from the thyroxine produced by the consumer's own thyroid gland. Thus, consumption of animal prey of any kind delivers both thyroxine and iodine into the bloodstream.

Although it would be useful to be able to estimate the iodine and thyroxine content of Neandertal prey, it is important to note that modern European animals (whether wild or domestic) are not appropriate analogues for late Pleistocene megafauna. Although many of the prey species that Neandertals regularly consumed (like the woolly rhinoceros and mammoth) are now extinct (Tattersall 1999), the iodine content of their tissues should have exceeded levels found in similar extant taxa. This is because the primary cause of the iodine-poor soils in Europe is leaching due to glaciation (Dobson 1998, 15), and one of the most extensive glacial events of the Pleistocene in that region came after the period of Neandertal occupation, not before (Tattersall 1999). This means that the recently glaciated soils responsible for the extraordinary incidence of iodine deficiency disorders and cretinism in modern and historic European human populations (Dobson 1998) could not have been as iodine-deficient during the time of Neandertal occupation. Thus, we have no reason to assume that prey animals consumed by Neandertals were an especially iodine- or thyroxine-deficient food source.

Modern humans have a very low tolerance of fluctuations in circulating thyroxine levels—both too little (hypothyroidism) and too much (hyperthyroidism) over an extended period make us decidedly ill. The rate of turnover for a measured amount of thyroid hormone in modern humans is very slow, about six and one-half days. In contrast, carnivores such as dogs have a thyroid turnover rate of about sixteen hours (Kaptein, Hays, and Ferguson 1994). Although dogs can suffer from too little thyroid hormone, they can ingest large amounts of exogenous hormone without problems (Ferguson 1994). Because Neandertals had a significantly higher component of raw meat in the diet than do modern humans, they must have had a higher turnover rate for thyroxine. A higher turnover rate for thyroxine means that a distinctly different pattern of thyroxine production must have existed for Neandertals.

More direct evidence on Neandertal thyroxine patterns comes from new chronological aging techniques that measure incremental growth lines in tooth enamel (perikymata). Aging studies on selected fossils suggest that Neandertals had faster postnatal growth rates than do modern humans (Stringer and Gamble 1993). Similar differences in early postnatal growth rates resulting in distinctive morphologies

are documented for dogs as compared with wolves, closely related animals with the same gestation period (Wayne 1986). Frank Williams, Laurie Godfrey, and Michael Sutherland recently examined craniofacial growth differences between Neandertals and anatomically modern humans and compared them with differences in another closely related species pair—the common chimpanzee and the pygmy chimpanzee or "Bonobo" (2002). Their study confirms a faster postnatal growth rate for Neandertals: Neandertals are morphologically distinct from modern humans from the early postnatal period onward, and these differences are at least as great as, or greater than, those manifested by different species of chimpanzees. Evidence of such a different postnatal growth rate for Neandertals is the most reliable indicator that they possessed a distinct pattern of thyroxine production.

The distinctive skeletal morphology possessed by Neandertals is almost certainly the result of a pattern of thyroxine secretion (and, therefore, of prenatal and postnatal growth rates) that differed markedly and consistently from that of modern humans. These Neandertal traits may resemble superficially the pathological changes associated with congenital iodine deficiency because they reflect different amounts of thyroxine available for skeletal growth at particular stages of development as compared with healthy modern humans.

I propose that the ancestors of Neandertals who chose to colonize arctic steppe environments initially consisted of a small founding population of individuals who were physiologically tolerant of the severe climatic conditions of that specific new environment. Only those colonizing individuals with the thyroxine phenotypes that gave them both cold tolerance and a high tolerance of a diet rich in raw meat would have been able to stay healthy enough to reproduce successfully. The reduced variation of thyroxine phenotypes manifested in this small population of incipient Neandertals resulted in the establishment of a new hormonal pattern in their descendants. The developmental and physiological consequences of this new hormonal pattern was the rapid generation of a new species, *Homo neanderthalensis.*

The evidence that Neandertals possessed a distinctive pattern of thyroxine production suggests strongly that they should be classified as a distinct species of *Homo* rather than a subspecies of *Homo sapiens.* On its own, this evidence is not conclusive, because we do not yet have a clear understanding of what levels of difference separate within-species variation from between-species variation for thyroxine production. Additional corroborative evidence, however, comes from comparative genetic studies. Analysis of mitochondrial (mt) DNA sequences suggests strongly that Neandertals were a genetically distinct lineage (Krings and others, 1997, 2000; Lindahl 1997; Ward and Stringer 1997; Ovchinnikov and others 2000). The combined evidence that they possessed distinct patterns of thyroid hormone production and were genetically distinct offers a strong, if not ironclad, case for making a taxonomic distinction between Neandertals and modern humans.

Reluctance to classify these hominids as distinct species is due in part to a misguided belief that this would necessarily rule out interbreeding between the two when declining Neandertal populations met increasing numbers of anatomically modern

humans. However, recent molecular data confirm that hybridization between closely related species is much more common than previously thought (Crockford 2000). Hybridization as an adaptive evolutionary strategy has been shown to be neither rare nor "unnatural" in animals, and hominids were not likely an exception (Arnold 1997). A few Neandertals could very easily have been assimilated into populations of anatomically modern humans, adding the genes for their unique thyroxine patterns to the *Homo sapiens* lineage, even if their distinctive mtDNA lineages were lost.

Other Examples: Incongruities between Morphological and Genetic Data

Recent studies suggest that a simple control mechanism for speciation, as proposed here, must exist to explain the close genetic relationship of species that otherwise possess distinct differences in life history, morphology, and behavior. For example, Antarctic Weddell and leopard seals are genetically more similar to each other than would be expected given the extent of differences in morphology and life-history traits between them (Arnason and others 1995). The thyroid rhythm theory predicts that when an ancestral species colonizes a radically new habitat with novel stress-producing characteristics, descendant species will be morphologically distinct but genetically similar until mutations have had a chance to accumulate.

Conversely, evidence that speciation events have occurred on more than one occasion from genetically distinct races of the same ancestral species (polyphyletic origins) have recently been reported in a number of taxa. Many of our domestic animals, for example, have been shown to descend from more than one geographical subspecies of the same ancestor (at least two in sheep, three in goats, four or more in cattle and dogs, and more than six in horses), multiple domestication events that produced essentially identical morphological results each time (Hiendleder and others 1998; Mannen and others 1998; Koop and others 2000; MacHugh and Bradley 2001; Vilà and others 2001; Jansen and others 2002).

The thyroid rhythm theory also offers a plausible explanation for polyphyletic evolution: When similar ancestors—closely related species or geographically isolated subspecies—colonize new habitats with identical or similar stress-producing characteristics, the descendants of each will be morphologically similar to each other but will retain the mtDNA distinctiveness of their respective ancestors. We should therefore expect to see different relationships among genetic and morphological characteristics, depending on the circumstances of the speciation event (the identity of the ancestor and the relative novelty of the new habitat—if the speciation event is the result of colonization, which is not always so). Morphological differences will not always be accompanied by genetic differences of the same magnitude, especially for mtDNA.

Conclusions

The thyroid rhythm theory summarized here provides a plausible explanation for the superficial resemblance of Neandertals to endemic cretins that is independent

of a pathological or iodine-dependent causality (Dobson 1998; Dobson and Geelhoed 2001). I suggest that the apparent similarity in skeletal structure between modern endemic cretins and European Neandertals is purely coincidental and is due to skeletal growth that is strongly controlled by species-specific thyroxine secretion. The suite of genes that generates species-specific thyroid rhythms have no influence on iodine uptake or storage capability. Selection for a particular, naturally occurring thyroid rhythm phenotype in Neandertal ancestors, when isolated within a small, interbreeding population of colonizers, could have quickly generated a new hominid species with distinctive skeletal characteristics. What makes this theory an appealing explanation, however, is that it clarifies much more than Neandertal morphology: It can be applied to speciation and adaptation events within the entire hominid lineage.

For example, the role of thyroxine in polyphyletic evolution explains how Neandertals and modern human groups such as Inuit and Lapps (Sami) could end up with similar skeletal morphology. Although the ancestors of each were genetically distinct, if closely related, species, colonization of both Pleistocene and Holocene arctic environments involved exposure to virtually identical physiological stresses. Because the underlying biological control mechanism for dealing with those stresses was the same for both groups, similar morphological consequences ensued. Critical differences among the ancestral populations mean that the resulting morphotypes were not truly identical, as Milford Wolpoff has noted, but noticeably similar (1999).

The thyroid rhythm theory also offers a plausible explanation for how our distinctly bipedal hominid lineage could have arisen in the first place and, perhaps most important, provides a new perspective on the plethora of thyroid deficiency disorders found in modern humans (Crockford, in press). Clearly, the concept that genetically determined thyroid rhythms constitute the biological mechanism that allows species to change and adapt over time has exciting implications for hominid evolution and health that go far beyond explaining Neandertal morphology.

References

Aiello, L., and C. Dean. 1990. *An Introduction to Human Evolutionary Anatomy.* London: Academic Press.

Arnason, U., K. Bodin, A. Gullberg, C. Ledje, and S. Mouchaty. 1995. A Molecular View of Pinniped Relationships with Particular Emphasis on the True Seals. *Journal of Molecular Evolution* 40 (1): 78–85.

Arnold, M. L. 1997. *Natural Hybridization and Evolution.* New York: Oxford University Press.

Arsuaga, J. L. 2000. The First Europeans: Spanish Caves Paint a New Picture of Evolution on the Continent. *Discovering Archaeology,* November/December, 48–65.

Balter, V., A. Person, N. Labourdette, D. Drucker, M. Renard, and B. Vandermeersch. 2001. Les Néandertaliens étaient-ils essentiellement carnivores? Résultats préliminaires sur les teneurs en Sr et en Ba de la paléobiocénose mammalienne de Saint-Césaire. *Comptes Rendus de l'Academie des Sciences de Paris, Sciences de la Terre et des planétes / Earth and Planetary Sciences* 332: 59–65.

Belyaev, D. K. 1979. Destabilizing Selection as a Factor in Domestication. *Journal of Heredity* 70 (5): 301–308.

Chadwick, D. J., and J. A. Goode, eds. 2000. *Mechanisms and Biological Significance of Pulsatile Hormone Secretion.* Chichester, England: John Wiley & Sons.

Clutton-Brock, J. 1992. The Process of Domestication. *Mammal Review* 22 (2): 79–85.

Coppinger, R., and R. Schneider. 1995. Evolution of Working Dogs. In *The Domestic Dog: Its Evolution, Behaviour, and Interactions with People,* edited by J. Serpell, 21–47. Cambridge, England: Cambridge University Press.

Crockford, S. J. 2000. A Commentary on Dog Evolution: Regional Variation, Breed Development and Hybridisation with Wolves. In *Dogs through Time: An Archaeological Perspective,* edited by S. J. Crockford, 295–312. Oxford: Archaeopress.

———. 2002. Animal Domestication and Heterochronic Speciation: The Role of Thyroid Hormone. In *Human Evolution through Developmental Change,* edited by N. Minugh-Purvis and K. J. McNamara, 122–153. Baltimore, Md.: Johns Hopkins University Press.

———. In press. Thyroid Rhythm Phenotypes and Hominid Evolution: A New Paradigm Implicates Pulsatile Hormone Secretion in Speciation and Adaptation Changes. *International Journal of Comparative Biochemistry and Physiology Part A.*

Darwin, C. 1859. *On the Origin of Species by Means of Natural Selection, or, The Preservation of Favoured Races in the Struggle for Life.* London: J. Murray.

———. 1868. *The Variation of Animals and Plants under Domestication.* London: J. Murray.

Dobson, J. E. 1998. The Iodine Factor in Health and Evolution. *Geographical Review* 88 (1): 1–28.

Dobson, J. E., and G. W. Geelhoed. 2001. The Châtelperronian/Aurignacian Conundrum: One Culture, Multiple Human Morphologies? *Current Anthropology* 42 (1): 139–140.

Ferguson, D. C. 1994. Update on Diagnosis of Canine Hypo-Thyroidism. In *Thyroid Disorders,* edited by D. C. Ferguson, 515–539. Philadelphia: Saunders.

Fleagle, J. G. 1999. *Primate Adaptation and Evolution.* 2d ed. San Diego, Calif.: Academic Press.

Gancedo, B., A. L. Alonso-Gomez, N. de Pedro, M. J. Delgado, and M. Alonso-Bedate. 1997. Changes in Thyroid Hormone Concentrations and Total Contents through Ontogeny in Three Anuran Species: Evidence for Daily Cycles. *General and Comparative Endocrinology* 107 (2): 240–250.

Geelhoed, G. W. 1999. Metabolic Maladaptation: Individual and Social Consequences of Medical Intervention in Correcting Endemic Hypothyroidism. *Nutrition* 15 (11–12): 908–932.

Gould, S. J. 1977. *Ontogeny and Phylogeny.* Cambridge, Mass.: Belknap Press of Harvard University Press.

Hadley, M. E. 1984. *Endocrinology.* Englewood Cliffs, N.J.: Prentice Hall.

Härlid, A., and U. Arnason. 1999. Analysis of Mitochondrial DNA Nest Ratite Birds within the Neognathae: Supporting a Neotenous Origin of Ratite Morphological Characters. *Proceedings, Royal Society of London, Series B* 266: 305–309.

Hayes, J. P., and S. H. Jenkins. 1997. Individual Variation in Mammals. *Journal of Mammalogy* 78 (2): 274–293.

Hayes, T. B. 1997. Hormonal Mechanisms as Potential Constraints on Evolution: Examples from the Anura. *American Zoologist* 37 (6): 482–490.

Hiendleder, S., K. Mainz, Y. Plante, and H. Lewalski. 1998. Analysis of Mitochondrial DNA Indicates That Domestic Sheep Are Derived from Two Different Ancestral Sources: No Evidence for Contributions from Urial and Argali Sheep. *Journal of Heredity* 89 (2): 113–120.

Jansen, T., P. Forster, M. A. Levine, H. Oelke, M. Hurles, C. Renfrew, J. Weber, and K. Olek. 2002. Mitochondrial DNA and the Origins of the Domestic Horse. *Proceedings of the National Academy of Sciences of the United States of America* 99 (16): 10905–10910.

Kaptein, E. M., M. T. Hays, and D. C. Ferguson. 1994. Thyroid Hormone Metabolism: A Comparative Evaluation. In *Thyroid Disorders,* edited by D. C. Ferguson, 431–463. Philadelphia: Saunders.

Koop, B. F., M. Burbidge, A. Byun, U. Rink, and S. J. Crockford. 2000. Ancient DNA Evidence of a Separate Origin for North American Indigenous Dogs. In *Dogs through Time: An Archaeological Perspective,* edited by S. J. Crockford, 271–286. Oxford: Archaeopress.

Krings, M., A. Stone, R. W. Schmitz, H. Krainitzki, M. Stoneking, and S. Pääbo. 1997. Neandertal DNA Sequences and the Origin of Modern Humans. *Cell* 90 (1): 19–30.

Krings, M., C. Capelli, F. Tschentscher, H. Geisert, S. Meyer, A. von Haeseler, K. Grosschmidt, G. Possnert, M. Pavnovic, and S. Pääbo. 2000. A View of Neandertal Genetic Diversity. *Nature Genetics* 26 (2): 144–146.

Kurtén, B., and E. Anderson. 1980. *Pleistocene Mammals of North America.* New York: Columbia University Press.

Lightman, S. L., R. J. Windle, M. D. Julian, M. S. Harbuz, N. Shanks, S. A. Wood, Y. M. Kershaw, and C. D. Ingram. 2000. Significance of Pulsatility in the HPA Axis. In *Mechanisms and Biological*

Significance of Pulsatile Hormone Secretion, edited by D. J. Chadwick and J. A. Goode, 244–260. Chichester, England: John Wiley & Sons.

Lindahl, T. 1997. Facts and Artifacts of Ancient DNA. *Cell* 90 (1): 1–3.

MacHugh, D. E., and D. G. Bradley. 2001. Livestock Genetic Origins: Goats Buck the Trend. *Proceedings of the National Academy of Sciences of the United States of America* 98 (10): 5382–5384.

Mannen, H., S. Tsuji, R. T. Loftus, and D. G. Bradley. 1998. Mitochondrial DNA Variation and Evolution of Japanese Black Cattle (*Bos taurus*). *Genetics* 150 (3): 1169–1175.

Manzi, G., A. Gracia, and J.-L. Arsuaga. 2000. Cranial Discrete Traits in the Middle Pleistocene Humans from Sima de los Huesos (Sierra de Atapuerca, Spain): Does Hypostosis Represent Any Increase in "Ontogenetic Stress" along the Neanderthal Lineage? *Journal of Human Evolution* 38 (3): 425–446.

Mayr, E. 1988. *Toward a New Philosophy of Biology: Observations of an Evolutionist.* Cambridge, Mass.: Belknap Press of Harvard University.

McKinney, M. L. 1998. The Juvenilized Ape Myth: Our "Overdeveloped" Brain. *BioScience* 48 (2): 109–116.

McNabb, A. F. M., and C. M. Wilson. 1997. Thyroid Hormone Deposition in Avian Eggs and Effects on Embryonic Development. *American Zoologist* 37 (6): 553–560.

McNamara, K. J., ed. 1995. *Evolutionary Change and Heterochrony.* Chichester, England, and New York: Wiley.

Mellars, P. 1996. *The Neanderthal Legacy: An Archaeological Perspective from Western Europe.* Princeton, N.J.: Princeton University Press.

Morey, D. F. 1992. Size, Shape, and Development in the Evolution of the Domestic Dog. *Journal of Archaeological Science* 19 (2): 181–204.

Ovchinnikov, I. V., A. Götherström, G. P. Romanova, V. M. Kharitonov, and W. Goodwin. 2000. Molecular Analysis of Neanderthal DNA from the Northern Caucasus. *Nature* 404 (6777): 490–493.

Reppert, S. M., and D. R. Weaver. 2002. Coordination of Circadian Timing in Mammals. *Nature* 418 (6901): 935–941.

Romer, A. S. 1970. *The Vertebrate Body.* 4th ed. Philadelphia: W. B. Saunders.

Schreibman, M. P., C. G. Scanes, and P. K. T. Pang, eds. 1993. *The Endocrinology of Growth, Development, and Metabolism in Vertebrates.* San Diego, Calif.: Academic Press.

Short, R. V., and E. Balaban, eds. 1994. *The Differences between the Sexes.* Cambridge, England, and New York: Cambridge University Press.

Stock, C. 1956. *Rancho La Brea: A Record of Pleistocene Life in California.* 6th ed. Science Series, 20 (Paleontology, No. 11). Los Angeles: Los Angeles County Museum.

Stock, C., and J. F. Lance. 1948. The Relative Lengths of Limb Elements in *Canis dirus. Bulletin of the Southern California Academy of Science* 47: 79–84.

Stringer, C., and C. Gamble. 1993. *In Search of the Neanderthals: Solving the Puzzle of Human Origins.* New York: Thames and Hudson.

Tattersall, I. 1999. *The Last Neanderthal: The Rise, Success, and Mysterious Extinction of Our Closest Human Relatives.* Rev. ed. Boulder, Colo.: Westview Press.

Tchernov, E. 1993. From Sedentism to Domestication: A Preliminary Review for the Southern Levant. In *Skeletons in Her Cupboard: Festschrift for Juliet Clutton-Brock,* edited by A. Clason, S. Payne, and H.-P. Uerpmann, 189–233. Bloomington, Ind.: Oxbow Books.

Trinkaus, E., and P. Shipman. 1993. *The Neandertals: Changing the Image of Mankind.* New York: Knopf.

Trut, L. N. 1999. Early Canid Domestication: The Farm-Fox Experiment. *American Scientist* 87 (2): 160–169.

Vilà, C., J. A. Leonard, A. Götherström, S. Marklund, K. Sandberg, K. Lidén, R. K. Wayne, and H. Ellegren. 2001. Widespread Origins of Domestic Horse Lineages. *Science* 291 (5503): 474–477.

Voss, S. R., and H. B. Shaffer. 1997. Adaptive Evolution via a Major Gene Effect: Paedomorphosis in the Mexican Axolotl. *Proceedings of the National Academy of Sciences of the United States of America* 94 (25): 14185–14189.

Ward, R., and C. Stringer. 1997. A Molecular Handle on the Neanderthals. *Science* 388 (6639): 225–226.

Waterman, A. J. 1958. Development of the Thyroid-Pituitary System in Warm-Blooded Amniotes. In *Comparative Endocrinology,* edited by A. Gorbman, 351–367. New York: John Wiley & Sons.

Wayne, R. K. 1986. Cranial Morphology of Domestic and Wild Canids: The Influence of Development on Morphological Change. *Evolution* 40 (2): 243–261.

Williams, F. L., L. R. Godfrey, and M. R. Sutherland. 2002. Heterochrony and the Evolution of Neandertal and Modern Craniofacial Form. In *Human Evolution through Developmental Change,* edited by N. Minugh-Purvis and K. J. McNamara, 405–441. Baltimore, Md.: Johns Hopkins University Press.

Windle, R. J., S. A. Wood, S. L. Lightman, and C. D. Ingram. 1998. The Pulsatile Characteristics of Hypothalmo-Pituitary-Adrenal Activity in Female Lewis and Fischer 344 Rats and Its Relationship to Differential Stress Responses. *Endocrinology* 139 (10): 4044–4052.

Wolpoff, M. H. 1999. *Paleoanthropology.* 2d ed. Boston: McGraw-Hill.

Wright, M. L. 2002. Melatonin, Diel Rhythms, and Metamorphosis in Anuran Amphibians. *General and Comparative Endocrinology* 126 (3): 251–254.

Appendix B: Published review paper,

Hominid evolution.

Crockford, S.J. 2003. Thyroid hormone rhythms and hominid evolution: a new paradigm implicates pulsatile thyroid hormone secretion in speciation and adaptation changes. ***International Journal of Comparative Biochemistry and Physiology Part A – Molecular & Integrative Physiology*** **135(1):105-129.**

ELSEVIER

Comparative Biochemistry and Physiology Part A 135 (2003) 105–129

CBP

www.elsevier.com/locate/cbpa

Review

Thyroid rhythm phenotypes and hominid evolution: a new paradigm implicates pulsatile hormone secretion in speciation and adaptation changes

Susan J. Crockford*

Pacific Identifications Inc, 6011 Oldfield Rd., R.R. 3, Victoria B.C., Canada V9E 2J4

Received 19 March 2002; received in revised form 15 July 2002; accepted 8 August 2002

Abstract

Thyroid hormones (THs, T_3/T_4) are essential central regulators that link many biological tasks, including embryonic and post-natal growth, reproductive function, and the behavioral and physiological responses to stress. Recently I proposed a novel theory to explain the role of THs in vertebrate evolution. Here I review the concept and discuss its ability to explain changes over time in hominid morphology, behavior and life history. THs are produced in a distinctly pulsatile manner and appear to generate species-specific TH rhythms with distinct ontogenic shifts. Individual variations in genetically controlled TH rhythms (TR phenotypes) must generate coordinated individual variation in morphology, reproduction and behavior within populations. Selection for any manifestation of a particular TR phenotype in an ancestral population selects all traits under thyroid control, resulting in rapid and well-coordinated changes in descendants. The concept provides the first really plausible explanation for a number of phenomena, including the convergent evolution of bipedalism in early hominids, species-specific sexual dimorphism, coordinated changes in morphology, brain function and gut length over time in hominids, cold adaptation in *Homo neanderthalensis*, the possible independent evolution of *H. sapiens* in Asia, and regional adaptation of hominid populations. This new paradigm provides a unique theoretical framework for explaining human origins that has important implications for human health.

Keywords: Evolution; Heterochrony; Speciation; Adaptation; Human origins; Pulsatile hormone secretion; Thyroid hormone; Thyroxine; Hypothyroidism

1. Introduction

Thyroid hormones from maternal sources are required by the human fetus for normal development. After birth, the rapid brain and skeletal growth that occurs during the early post-natal period are also dependent on relatively high levels of available thyroid hormones (Braverman and Utiger, 1991; Hauser et al., 1998; Geelhoed, 1999; Cudd et al., 2002). This reliance on thyroid hormones for normal growth is characteristic of all vertebrates, however, not just humans (Hadley, 1984). While the essential nature of thyroid hormones in development is well recognized, the evolutionary significance of this fact has been virtually ignored.

Within vertebrates, traits that reflect recognizable differences in growth and developmental are commonly noted both within and between species.

*Tel.: +1-250-721-7296; fax: +1-250-721-6215.
E-mail address: scrock@tnet.net (S.J. Crockford).

1095-6433/03/$ - see front matter
PII: S1095-6433(02)00259-3

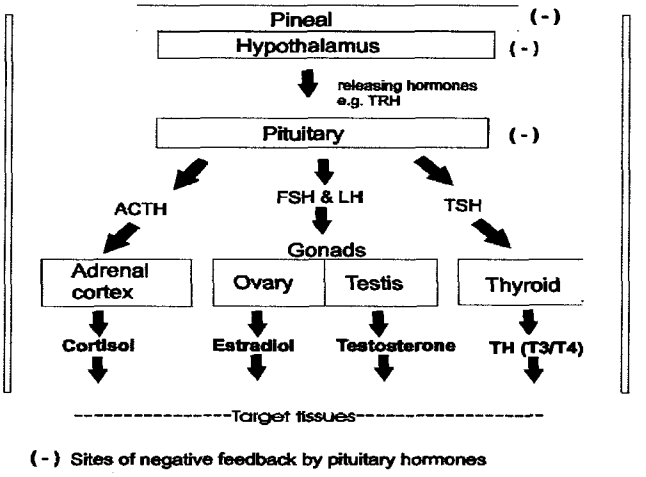

Fig. 1. The classic model of pituitary hormone function (after Hadley 1984: 129) that misrepresents the importance of THs; see Fig. 2 for definitions.

Changes in the rates and timing of both embryonic and post-natal development in ancestral species make possible a wide variety of size and shape differences in descendant populations, a well-known evolutionary pattern known as heterochrony. Heterochrony has been identified as the most common mode of speciation in virtually all lineages (Gould, 1977; McNamara, 1995; McNabb and Wilson, 1997; Yeh, 2002), although how these changes are implemented is still unclear. Much evolutionary research has focused on the role of so-called *Hox* genes in the initiation of early embryonic developmental change (Barres et al., 1994; Krumlauf, 1994; Lawrence and Morata, 1994). Thyroid hormones, along with retinoic acid (a derivative of vitamin A), have been identified as essential for the expression of many *Hox* genes (Hayes, 1997; Voss and Shaffer, 1997; McKinney, 1998), and yet a pivotal role for thyroid hormones in regulating development seems not to have been considered.

Although the vertebrate thyroid gland produces hormones in several forms, the principal two are T_3 (triiodothyronine) and T_4 (thyroxine). While both forms are secreted by the thyroid gland, conversion to T_3 via deiodination from T_4 also occurs in blood and tissues. T_3 has a shorter half-life and is more metabolically active than T_4 (Hadley, 1984). T_3 may be more involved in gene regulation and T_4 more important in development (Brent et al., 1991; Brent, 2000), although in many instances it is unclear which form is involved. The term 'thyroid hormones' (THs) is thus used here to refer to both forms together, and T_3 or T_4 to refer to each form specifically.

THs by themselves control both cellular and basic metabolic rate, early embryonic cell migration and differentiation, and the expression of a number of strategic genes (see Crockford, 2000a, 2002a for more details). They are essential for fetal development and post-natal growth of the entire central nervous system (CNS), including the eyes and brain (Schreibman et al., 1993). In the digestive system, THs are known to be responsible for differentiation of the epithelial lining of the small intestine (where nutrient absorption occurs) and to affect the timing of tooth eruption (Risinger and Proffit, 1997) and tooth enamel formation (Noren and Alm, 1983; Koyama et al., 2001).

THs also function as essential co-factors in the production and utilization of other critical hormones, and thus control function of the brain, reproductive organs, adrenal glands and hair follicles (Hadley, 1984). In addition, THs control release and utilization of growth hormones, which stimulate the liver to release insulin-like growth factor-I (IGF-I), an important hormone shown to vary in different human and ape populations (Shea, 1992; Davilla et al., 2002). However, traditional models of hormonal cascades that neatly flow from top to bottom (Fig. 1) are so simplified that they misrepresent the relationship of thyroid hormones to the others. In reality, the production and actions of these hormones operate more like the model

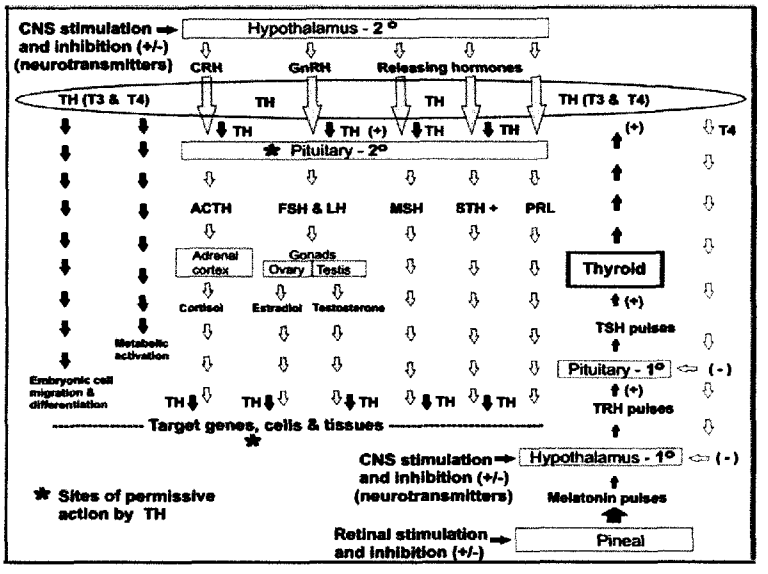

Fig. 2. A model that accurately depicts the role of THs on the actions and effects of pituitary hormones. TH affects embryonic growth and metabolism directly, while other systems are permissively affected. A primary release of TH (*far lower right*) precedes a secondary release of hypothalamic and pituitary hormones. The pool of THs thus created (*top*) is permissively required (indicated by D) for both the release of pituitary hormones and their subsequent affects on target genes, cells and tissues. CNS, central nervous system; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone (or thyrotropin); GnRH, gonadotropin-releasing hormone; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropin (corticotropin or adrenal cortical-stimulating hormone); FSH, follicle-stimulating hormone (or follitropin); LH, luteinizing hormone (or lutropin); MSH, melanocyte-stimulating hormone; STH, somatotropin (or GN, growth hormone, which stimulates release of insulin-like growth factor-I or IGF-I from the liver); PRL, prolactin.

shown in Fig. 2, where flow starts from the bottom. THs must be present for both pituitary and adrenal hormones to be secreted and to exert their effects on tissues. Therefore, one of the most important roles of THs is to ensure that all hormone-producing organs give a coordinated response to changing physical, physiological and psychological conditions (Hadley, 1984).

The release of THs into the bloodstream is distinctly pulsatile in nature due to the intermittent stimulus for TH secretion that the gland receives from the pituitary (via thyrotropin or thyroid-stimulating hormone, TSH). This pulsatile pattern of TH production is its most critical characteristic. Pulsatility originates high in the hormonal cascade, where electrical stimulation of receptors in the retina and CNS relay signals to the pineal gland. The pineal gland translates these electrical signals into a biochemical message, principally the neurohormone melatonin (Korf, 1994). Pulsatile release of pineal melatonin (Haisenleder et al., 1992; Wright, 2002) stimulates pulsatile secretion of thyrotropin-releasing hormone (TRH) from the hypothalamus. TRH pulses stimulate bursts of TSH from the pituitary, and TSH pulses finally stimulate pulsatile release of THs from the thyroid glands.

Once THs have been released, they are available for a number of body functions, via both direct and permissive actions (Fig. 2). Many researchers seem to have lost sight of this relationship altogether, as observed in the otherwise fascinating volume edited by Chadwick and Goode (2000). Incredibly, in this compilation of papers on the biological significance of pulsatile hormone secretion, THs are not ever mentioned. Since pituitary hormones are so dependent on THs for their release, the fact that THs are also produced in a pulsatile fashion (Bitman et al., 1994; Gancedo et al., 1997; Wright, 2002) suggests that THs may be the pacemaker that drives pulsatility in the others.

The reason that THs are so strongly implicated in evolutionary change is not just due to their crucial role in both embryonic development and post-natal growth, also because they are the only factor that links essential morphological, physiological and behavioral traits.

In the course of a critical re-examination of domestication as an evolutionary process, I developed a theory to explain the role of THs in heterochronic speciation events for all vertebrate taxa (Crockford, 2002a). The theory states that the biological mechanism responsible for generating new species—reproductively isolated descendant populations with a distinctive suite of well-coordinated traits—involves selection for particular variants of TH production patterns (thyroid rhythm phenotypes) that occur naturally within ancestral species. Here I review the concept using hominid evolution as an especially interesting example.

A new perspective such as this is long overdue in evolutionary biology. It provides a precise biological mechanism to account for evolutionary change and is a powerful theory based on explicit biological hypotheses that can be tested experimentally. As a consequence, the concept provides a strong theoretical framework for future research. It constitutes a significant paradigm shift (Kuhn, 1970) because it looks at the question of how species evolve from a completely different direction than the population genetic models that have dominated the field until now (reviewed in Mayr, 1982; Schwartz, 1999). In addition, the theory more accurately reflects and predicts the complex nature of inter- and intra-specific relationships we are now able to discern using phylogenetic analysis methods (e.g. Arnason et al., 1995). Perhaps most astonishing is the ability of this new concept to make evolution personal—something the old paradigm could never do. Examining the potential role of THs in hominid evolution provides crucial insight into understanding how THs affect the health of each and every one of us as we grow, reproduce and age.

2. Thyroid hormones, heterochronic speciation and adaptation

Due to their central regulatory role and pleitropic actions, THs are strongly implicated in the mechanism that controls both rapid speciation changes and gradual adaptation of populations over time. During pulsatile TH secretion, some precision in the timing (frequency of pulses) and absolute amounts of hormone produced (amplitude of pulses) are almost certainly crucial to target genes, cells and organ tissues that depend on THs. Blood concentrations of THs are known to change in particular ways with age and season, and in response to other physiological demands, such as reproductive stage (especially in females), psychological state and general health (De Pablo, 1993; Kaptein et al., 1994), changes that likely reflect shifts in the frequency and intensity of TH pulses. More significantly, the few comparative studies undertaken so far indicate that basic daily rhythms of TH production are species-specific.

For example, Gancedo et al. (1997) measured THs four times a day in larvae of three anuran species with different phylogenetic origins, behaviors and ecological habits (*Rana perezi*, *Xenopus laevis*, *Bufo calamita*). They found that daily profiles of both T_3 and T_4 differed significantly between taxa and during ontogenic development for all three species. Similarly, in birds it has been found that the pattern of post-hatching rise in TH levels is significantly different between neonates of species that produce precocious young (European starlings) and those with altricial young (Japanese quail and ostrich) (Schew et al., 1996; Härlid and Arnason, 1999). Due to the permissive actions of THs, the many studies that document individual variation and breed differences in rhythms of other hormones (including growth, reproductive and adrenal hormones) provide indirect evidence of TH pulsatility (Windle et al. 1998; Butler, 2000; Lightman et al., 2000; Schaefer, 2000; Velduis, 2000).

Differences in overall TH metabolism have been demonstrated in a number of closely related taxa, although many simply compare single-point concentrations that could result in sampling underlying species-specific rhythms at different points. Lapseritis and Hayssen (2001), however, not only found distinct differences in T_4 levels between two subspecies of deer mouse (*Peromyscus maniculatus*), but between each of their agouti and non-agouti color morphs, a study that confirmed other reports of TH function differences in these taxa. Gagneux et al. (2001) recently reported that one of the few genetic differences between modern *Homo sapiens* and their closest living relatives, *Pan troglodytes* (chimpanzee) and *P. paniscus* (bonobo), is an approximately two-fold higher level of transthyretin (a molecule that transports TH) in the blood and cerebrospinal fluid in *Pan*, a finding in addition to data that documented that both free (unbound) and total THs differed significantly between the genera.

Examining turnover rates of T_4 is one way of reliably comparing thyroid function between spe-

cies, and although it still does not reflect pattern differences, it at least avoids equating potentially ambiguous single-point blood concentrations. For example, the average half-life of T_4 has been found to be 13 h in dogs, 16.6 h in cats and 6.8 days in humans (Kaptein et al., 1994). Differences in turnover rates between domestic animal breeds have also been demonstrated: for example, the half-life of T_4 in the beagle (a dog breed that is typically diestrous) is twice that of the basenji (a breed that is typically monoestrous) (Nunez et al., 1970). Clearly, while few studies have yet been carried out that are appropriate for making broad comparisons within and between species for TH rhythms, there have been enough to suggest that significant differences exist.

Because growth and development are so dependent on available THs, I suggest that species-specific (and breed-specific) TH rhythms must control species-specific (and breed-specific) growth. While mutations in receptor genes (and their co-factors) and the relative availability of TH transport molecules may modify this control by changing the utilization efficiency of THs at the tissue level, rhythm differences are almost certainly the most significant factor regulating species-specific development. In addition, subsequent interactions of species-specific TH rhythms with both growth and sex hormones provide the first truly plausible explanation for how species-specific sexual dimorphism is achieved (Short and Balaban, 1994; Schwartz and Dean, 2001; German and Stewart, 2002).

Slight mutations in one or several of the genes responsible for the pulsatile release of THs are bound to occur, and these mini-mutations (along with recombination that occurs during fertilization) generate slight, individual rhythm differences within species. If precision in timing and absolute amounts of THs produced are critical to different tissues at different ontogenetic stages, then even slight variations in the rhythm of hormone production would result in small physiological differences between individuals or thyroid rhythm (TR) phenotypes. While genes at other sites may modify the ultimate rhythmic pattern of THs, output of several so-called 'clock genes'—found in circadian oscillator cells that reside in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus—are likely the origin of pulsatile production (Reppert and Weaver, 2001, 2002). Even very slight individual variation in the efficiency of such genes (eight identified so far) may have dramatic repercussions for traits downstream through their affects on secretion of the pineal gland hormone melatonin (Wright, 2002).

I suggest that because THs influence so many traits and body functions, individual TR phenotypes must underlie many of the morphological, reproductive and behavioral differences we recognize as individual variation within a species or domestic breed (Gould, 1977; Hayes and Jenkins, 1997; Windle et al., 1998; Lightman et al., 2000; Grant and Grant, 2002). In other words, many seemingly unrelated morphological, physiological and behavioral traits are not controlled by individual variable genes that act and inherit independently but by the small suite of tightly linked genes that exert their effects indirectly through control over the TR phenotype of each individual. Thus, selection for any one trait controlled by the TR phenotype also selects for all other traits linked to it by the physiological network, allowing morphology, behavior and life history strategies to change together in a highly coordinated fashion. As a consequence, TR phenotypes are postulated as providing the essential raw material—the individual variation—for natural selection to act upon during adaptation and speciation (Darwin, 1859, 1868; Mayr, 1988).

The intimate role that THs play in the response of animals to stress is pivotal to this concept. When populations expand their boundaries, new habitats offer many attractive benefits, but they also present stressful conditions for each colonizing individual. Novel habitats are postulated as preferentially attracting physiologically stress-tolerant individuals (those with particular TR phenotypes) over less stress-tolerant animals (see Crockford 2000a; Crockford, 2002a for more details). As colonizers constitute only those individuals with stress-tolerant physiological phenotypes, founder populations possess a non-random subset of the TR phenotypes (and the genes that produce them) maintained by ancestral populations. Variation of TR phenotypes (and the genotypes they represent) within such small founder populations will always be much smaller than that which existed in the ancestral population as a whole.

Due to the essential developmental role of THs, there would almost certainly be immediate consequences for offspring that result from matings within small and isolated founding populations

that have limited variation in TR phenotypes. Rapid changes in morphology would be expected to occur in descendant populations as a new hormonal rhythm for the group becomes established. Most importantly, changes to behavior and reproduction would occur simultaneously, as the entire hormonal cascade is impacted by the TR shift. Experimental domestication studies suggest that profound changes in behavior, reproductive physiology and morphology may occur within 20 generations under artificial selection for a stress-tolerant behavioral phenotype alone (Belyaev, 1979; Coppinger and Schneider, 1995; Trut, 1999). If natural selection takes even 10-fold as long (i.e. ca. 200 years) to effect the same result, it would still be almost instantaneous in evolutionary terms—certainly too fast for paleontologists to pick up intermediate stages in the fossil record.

Under these circumstances, colonizers of new habitats become morphologically, reproductively and behaviorally distinct so rapidly that they retain a very close genetic relationship to their ancestors. Nevertheless, a descendant population resulting from such a colonization event represents a new species as soon as a unique TR becomes established, and once this speciation change occurs, it is permanent as long as reproductive isolation is maintained. Behavioral and reproductive timing changes that occur as a consequence of the TR shift, in addition to the ecological partitioning that precipitated the event in the first place, tend to keep the new population reproductively discrete.

There is no evidence so far to suggest that any mitochondrial DNA haplotypes are associated with particular TR phenotypes. Thus, by chance, even a small colonizing population of stress-tolerant individuals may possess all (or nearly all) of the mtDNA variation that existed in the ancestral population. This explains many examples of the extremely close mtDNA relationship of many taxa that by all other measures are considered as fully distinct species. Such is the case for the polar bear (*Ursus maritumus*), a species so genetically close to one particular population of brown bear (*U. arctos*) that all phylogenetic analyses place them within the brown bear clade (Talbot and Shields, 1996). Distinctive polar bear morphology, physiology and life history traits appear to have evolved relatively recently—and rapidly—in the late Pleistocene (Fig. 3).

As stated previously, stress is an essential feature of virtually all speciation events. Stress is a ubiquitous factor that can have purely physical manifestations (such as light or temperature), or involve a psychological or behavioral component (such as dealing with predators, new food sources or competing for breeding sites) (Hadley, 1984). Existing variation in physiological or behavioral tolerance to stress could thus lead to the non-random subdivision of any population in response to changes in any stress-inducing condition.

After initial colonization events separate a few stress-tolerant individuals from an existing population, the particular circumstances of each new habitat present specific challenges to the founding population. Such challenges would impact the first few generations of founders heavily, leaving only those able to stay healthy enough to reproduce successfully as contributors to the next generation. The resulting changes in the descendants will vary according to the physiological make-up of each colonizing population, its size and the severity of the selection pressure. However, this concept supplies the first really plausible explanation for how multiple speciation events (taxa with polyphyletic origins, or so-called 'sibling species'; Mayr, 1982, 1991) could occur. Given virtually identical ancestral populations (such as populations of geographically isolated subspecies) and similar or identical new habitats available for colonization, virtually identical biological changes would be expected to occur. If the geographic subspecies in question have been isolated long enough for them to have developed some level of genetic distinctiveness, the species each population generates will be genetically distinct, despite the extreme similarities in morphological, behavioral and life history traits. The evidence is very strong that this is precisely what has occurred in the evolution of many domesticates. Mitochondrial DNA studies on cattle, sheep, goats, dogs and horses support previous suggestions that at least two, and in some cases four or more, separate domestication events have been involved (Hiendleder et al., 1998; Mannen et al., 1998; Koop et al., 2000; Luikart et al., 2001; MacHugh and Bradley, 2001; Jansen et al., 2002).

Similarly, the concept explains how convergent evolution could easily occur. When similar species are exposed to very similar climatic or habitat changes, we would expect similar kinds of changes to result, because the thyroid control mechanisms are the same and would respond in a similar manner. The resulting organisms will be genetical-

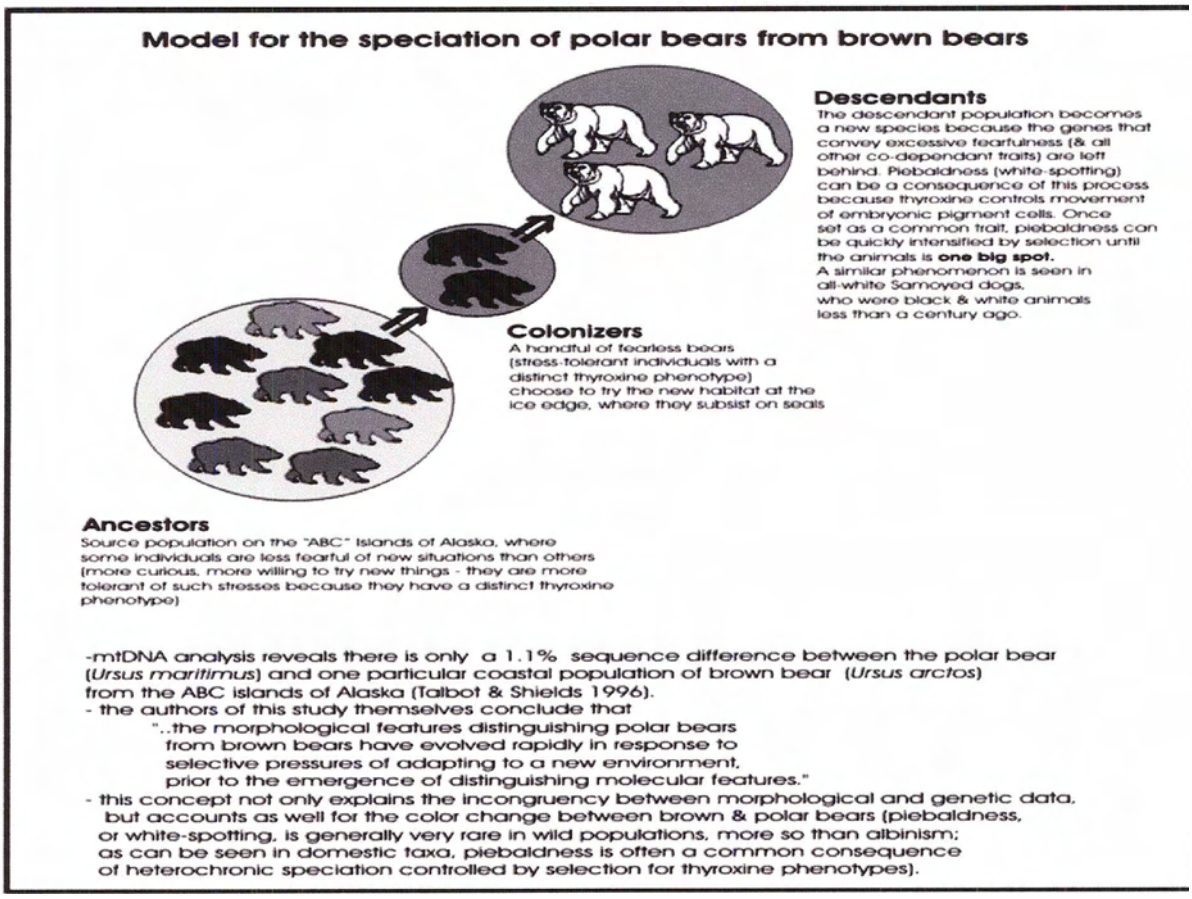

Fig. 3. A geographic model for heterochronic speciation through colonization of new habitats, using the polar bear as an example. Individuals with a relatively high tolerance to stress disperse preferentially over animals with less stress tolerance. Colonizing groups are thus composed of individuals that possess a limited range of TR phenotypes compared to their ancestors. New species result when a new TH rhythm is established, one different from the ancestral pattern.

ly distinct but similar in morphology—in some cases the similarities are so strong that they make sorting out evolutionary relationships extremely difficult. Numerous examples of these phenomena are now known (e.g. Arnason et al., 1995; Crockford, 2000b, 2002b) and the list is expanding rapidly.

The discussion so far has been limited to explaining speciation events, although a similar mechanism also explains adaptation of species over time. New conditions can be imposed on an entire population by sudden climatic shifts, for example. Only those individuals who already possess the traits (controlled by underlying TR phenotypes) that allow them to survive the changes and reproduce successfully despite them, pass their genes on to future generations. Thus, some TR phenotypes may decrease in frequency or be lost because those individuals die or do not reproduce, while other TR phenotypes increase in frequency.

An example of how rapidly adaptation can occur was reported recently by Grant and Grant (2002) in a rare, long-term investigation of two species of Galapagos finches. In their study, changes in the food supply over a 30-year period (brought about by alternating periods of drought and flood) selected several times for beaks of significantly different shapes and sizes. [Note that beak size and shape are traits set during early development and are thus almost certainly controlled by a species-specific TH rhythm, with slight individual variations provided by distinct TR phenotypes.] I

suggest this case demonstrates that even over the short term, individual variation in TR phenotypes provide the plasticity that allows frequencies of certain phenotypes in a population to shift rapidly enough in response to changes in environmental conditions that overall population survival is ensured.

If variation becomes much reduced in an adapting or colonizing group, however, how does it later increase so that adaptation can continue? It is possible that the genes controlling TR phenotypes in the SCN accumulate mutations at a fairly rapid rate, gradually increasing variation lost during a population crash. Given that there are many genes for which combined actions generate TR phenotypes, and that each individual gene within that complex cannot be directly subject to selection (since it is the product of the group as a whole—the TH rhythm—that is selected, via its downstream affects on physiological and physical traits), the individual genes that produce TR phenotypes may undergo slight mutations in virtually neutral fashion, creating a multitude of alleles. Such minimutations would ensure that individual differences in TH rhythms are constantly replenished.

In essence, I propose that populations adapt to changing environmental conditions over geological time using the same biological mechanism that individuals use to adapt to daily and seasonal changes. The concept offers the first really plausible explanation for how sexual dimorphism, polyphyletic origins and convergent evolution are achieved, and thus provides an especially useful framework for examining hominid evolution.

3. The first hominids

The earliest hominids are the Australopithecines, bipedal ancestors that appear in the fossil record at ca. 4.4 million years ago (mya). The pelvic, vertebral and femoral shape changes that allowed bipedal locomotion in Australopithecines preceded other morphological characteristics that make later hominids unique (Fleagle, 1999). What could have initiated such particular morphological changes in the first place? Various suggestions have been advanced, most of which assume that bipedal morphology arose because it conferred some survival or reproductive advantage in and of itself (such as for carrying infants or food).

However, bipedal morphology (or a bigger brain, or a shorter gut) does not arise because it would be advantageous to survival. Evolution is not a mail-order catalogue: natural selection can act only on traits that are already present within a population and they must convey either distinct survival advantages or disadvantages (Mayr, 1982). This means that some incipient hominids had to have had bipedal morphology, while others did not. The analysis by Berge (2002) confirms that bipedal traits are heterochronic in nature: they were caused by changes in developmental rates or shifts in timing of growth spurts. This strongly suggests that a heterochronic speciation event precipitated the changes associated with bipedalism, a speciation event that was very likely associated with colonization of a distinctly different habitat.

Australopithecines are first found associated with faunas typical of closed woodland forests, some broken by mixed bush (Reed, 1997). I suggest that Australopithecines evolved with a novel bipedal morphology because environmental changes pressured some of their ancestors to colonize a wooded habitat in which the prevalent foods were not the fruits they were accustomed to for eating, but small animals: insects and grubs, bird eggs and fledglings, small mammals, reptiles and amphibians. The dietary change associated with this habitat shift would have been profound, because it involved the consumption of vastly increased amounts of exogenous THs. Small prey animals, such as rodents, reptiles, amphibians and young birds, are generally eaten whole, which means that their thyroid glands (which contain especially high concentrations of THs) are consumed as well. Egg yolks of all vertebrates also contain THs (McNabb and Wilson, 1997).

THs are the only hormones that are absorbed unaltered through the digestive tract (Hadley, 1984), and exogenous THs from food sources are indistinguishable from self-produced hormone. Animals that have always been carnivores appear to handle massive influxes of exogenous THs without problems (Kaptein et al., 1994). However, herbivores or fructivores, even if they occasionally ate small animals, would have possessed a TH metabolism unprepared for such excess. Consumption of large quantities of TH-laden foods (rather than occasional small amounts), day after day and month after month, would have had a major impact on populations of incipient Australopithecines.

I suggest that only those individual Australopithecine ancestors who were relatively tolerant of high stress situations would have chosen to colo-

nize a radically new environment in the first place. Experimental domestication suggests this stress-resistant component could comprise as much as 20% of the existing population (Belyaev, 1979). Even stress-tolerant colonizing individuals, however, would have varied somewhat in their ability to accommodate a dramatic change in food resources without major disruption of their reproductive potential.

In experimental animals

> There is abundant evidence that exogenous thyroxine crosses the placenta...and severe chronic hyperthyroidism [over-production of THs] of the pregnant rat produced cretinoid progeny (Waterman, 1958: 364–365)

Significant changes in normal TH levels during pregnancy (either too much or too little) certainly have profound affects on the developing fetus of modern humans (Porterfield, 1994; Weetman, 1997; Arem, 1999; Cudd et al., 2002). There is no reason to expect that incipient Australopithecines would have responded differently to consumption of exogenous THs that far exceeded their normal intake. It is very probable that the major shift in diet proposed for incipient Australopithecines, made necessary by the change in habitat, would initially have resulted in some instances of reduced fertility (failure to ovulate or conceive, repeated miscarriages or stillbirths) and a relatively high incidence of birth anomalies of various kinds. Offspring afflicted with profound anomalies probably died young. However, survival rates of infants with relatively minor anomalies, such as a change in pelvic and femoral shape that allowed them to stand upright with ease, may have been quite high. As long as such morphological changes did not negatively impact the survival of afflicted individuals, they would have had a reasonable chance of living to sexual maturity and passing on their genes to the next generation. If we view bipedalism as something akin to an unavoidable birth anomaly that could be adapted to behaviorally, it gives quite a new perspective to hominid evolution.

Incipient Australopithecine offspring with new, viable morphologies were generated because their mothers possessed the genes for a particular TR phenotype that was not totally disrupted by a diet high in exogenous THs. This resilient TR phenotype allowed almost-normal fetal development to proceed, generating offspring with relatively minor morphological differences that must have been accompanied by some behavioral and reproductive physiology differences. Bipedal offspring would have had a good chance of having a TR phenotype similar to their mother, and thus would have been likely to produce bipedal infants themselves. Over the next few generations, the specific growth programs that produced bipedal morphology would have become the norm for the whole founding population. Colonization of a radical new habitat and the associated dietary switch it necessitated undoubtedly precipitated the expression of several new morphotypes, but natural selection was responsible for the fact that bipedalism was the option that survived over time.

Support for this exogenous TH explanation comes from isotope analysis of *Australopithecus africanus* fossils from South Africa, which indicate that these first hominids consumed not fruits and leaves as often assumed, but animal prey that ate grasses (Sponheimer and Lee-Thorpe, 1999). These Australopithecines generated isotope signatures that were more similar to carnivorous hyenas than to any other herbivorous grazers or browsers of their time. *Paranthropus* fossils apparently show very similar patterns. Ironically, while the authors of this study concluded that "we must seriously consider the possibility that these hominids were ^{13}C-enriched because they consumed animal foods" (Sponheimer and Lee-Thorpe, 1999: 369), they offer only insects (termites) or the young of grazing bovids as possible choices. What about snakes that consumed grass-eating rodents, or the rodents themselves? What about insect-eating amphibians or small primates? Have we become so fixated on late hominid big-game hunting skills that we can consider no other prey items for their ancestors?

An exogenous TH explanation for the generation of bipedal morphology raises the question of whether bipedalism is exclusive of the hominid lineage. If the claims of bipedal morphology possessed by specimens of 4 400 000-year-old *Ardipethecus ramadens* (Fleagle, 1999), a newly described Australopithecine genus from east Africa at 3.5 mya (Leakey et al., 2001), and an even older new genus from 5–6 000 000-year-old deposits (*Orrorin tugenensis*) hold up, we will have to revise the date for the origins of hominids further still (Lieberman, 2001). The question is whether any of these genera, rather than *Australopithecus*, are ancestral to the line that later produced *Homo sapiens:* do some specimens represent cases of convergent evolution? If climatic change

at the end of the Miocene (at ca. 5 mya) resulted in a reduction of fruit-bearing trees in some regions of Africa—trees that had supported Miocene apes for thousands or millions of years (Kelly, 2002)—more than one ape lineage may have been forced to assume a diet heavily dominated by small animals. Consumption of high levels of exogenous THs would have had similar effects on any ape population as that described for Australopithecines: not identical, but similar enough to cause us confusion in sorting out the scanty fossil remains of closely related lineages. Just because *Orrorin* was bipedal does not automatically make it a human ancestor. The same could also be said for Australopithecus, but for the sake of this argument, I retain the currently accepted scenario.

In summary, I maintain that Australopithecine ancestors had to have been much more omnivorous than we have assumed, with small animals a minor but consistent part of their diet. Incipient Australopithecines later became bipedal apes with slightly larger brains as a direct result of a significant increase in their consumption of whole small animals. Population growth of this successful lineage would have lead to further subdivisions (additional species) associated with expansion of territories and local dietary specializations. For example, fossils of several species of the late robust Australopithecine genus, *Paranthropus*, are associated with wet grassland faunas (Reed, 1997). Such a habitat suggests *Paranthropus* perhaps had access to a greater range of small animal species, which may have included animals such as ground-nesting waterfowl, small aquatic mammals, turtles, slow-moving/shallow-dwelling fish, crayfish and shellfish. These items, especially eaten whole (including the bones), would likely have been as tough to chew and as abrasive as hard nuts and seeds, which could account for wear patterns found on their fossil teeth (Sponheimer and Lee-Thorpe, 1999).

The dietary shift proposed above for *Paranthropus*, from consumption of quite small animals to larger and more varied ones (including things with shells and spines), seems a likely stimulus for the manufacture of simple cutting or crushing tools. Thus, we might expect to observe some evidence of first simple tool use at this stage, and indeed this seems to be the case. Morphology of hand and foot remains of *P. robustus*, for example, suggested to Susman (1994) that they had the manipulative ability to have used tools, although solid evidence for tool use by *Paranthropus* is still lacking at this point.

Homo habilis retains the title of first hominid tool user. *H. habilis* appears to have diverged from *P. boisei*, *A. afarensis*, or one of their close relations at the beginning of the Pleistocene (Fig. 4). Fossils of this species are associated with somewhat drier, more open habitats, where bovid species were plentiful (Reed, 1997). Although these first members of the genus *Homo* likely scavenged carcasses of large species rather than actively hunted them, long-term survival in this new environment would have required incipient *H. habilis* colonizers to forage in exposed habitats where carcasses and predators abounded: that is, they had to possess TR phenotypes that were tolerant of the stresses inherent to open habitats.

Since the dietary changes faced by the first incipient *Homo* species were not nearly as large as those faced by incipient Australopithecines, we would not expect the morphological changes associated with these speciation events to be as dramatic, and indeed they are not. *H. habilis* was larger than any Australopithecine, with a slightly larger brain. However, other changes are so minor that some authors argue for placing *H. habilis* in the genus *Australopithecus*.

New developmental programs generated by recombination and shifts in proportions of the most common TRs in emerging *H. habilis* must have affected brain development in such a way as to confer slightly more dexterity (for tool manufacture and use) and perhaps better decision-making skills on this species, neural re-arrangements that gave it definite survival advantages in a more exposed habitat. Although *H. habilis* probably still ate some small animals and vegetation, their diet appears to have included a substantial amount of marrow and brains from scavenged carcasses (Fleagle, 1999). Such a diet would have been proportionally higher in the essential fatty acids necessary for brain development and function (Horrobin, 1997, 2001; Horrobin and Bennett, 1999).

However, organ tissues (including thyroid glands) are almost always consumed by the primary hunters of any prey and are not usually available to scavengers. Consequently, *H. habilis* (and similar species of that era, such as *H. rudolfensis*), even if a modest proportion of their diet

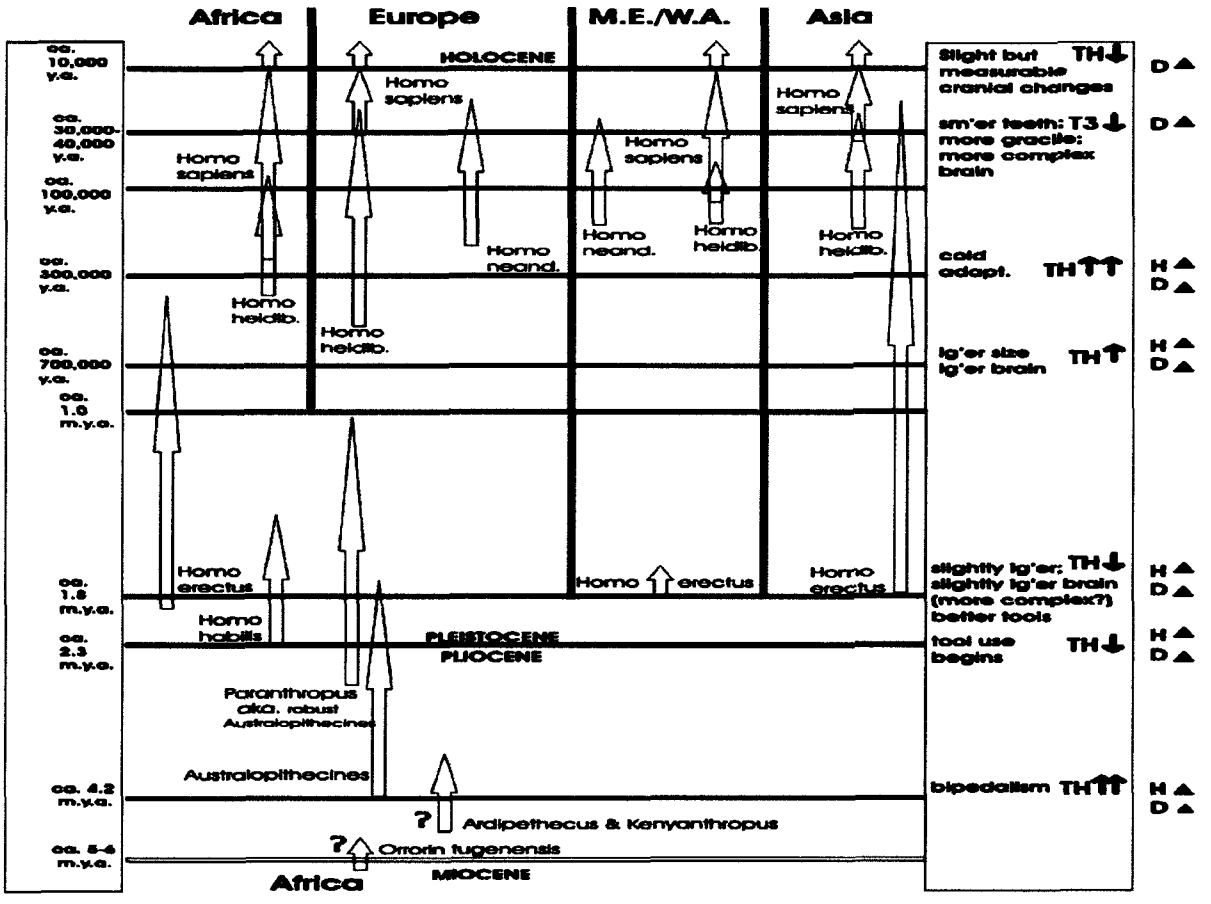

Fig. 4. Summary of hominid evolution, showing the regional location of significant fossils and the morphological changes they document. H▲ indicates a habitat change, D▲ indicates a diet change; TH↑ and TH↑↑ indicate slight and profound increases in dietary THs, respectively; TH↓ and T3↓ indicate decreases in THs and T3, respectively; M.E./W.A. is Middle East/Western Asia.

still consisted of small whole animals, must have consumed smaller quantities of exogenous THs than their Australopithecine ancestors. Selection would thus have favored individuals whose thyroid glands could produce adequate amounts of THs in the absence of exogenous sources.

If THs were at times available in less than optimal amounts for incipient *H. habilis*, especially for the brain development of offspring and the mental function of adults, a proportional excess of essential fatty acids (especially eicosapentaenoic acid, EPA; arachidonic acid, AA; and docosahexaenoic acid, DHA) may have compensated by making the most of what THs were available. Brain tissue is composed of eight percent each of AA and DHA (by dry weight), with lesser amounts of EPA: DHA and AA seem to be more involved in brain formation and structure while EPA is essential for brain function (Horrobin, 2001). We also know now that DHA is required for the production of transthyretin (Kitajka et al., 2002), a TH transport molecule that is particularly important for moving THs around the brain, and has a critical role in vertebrate brain development (Schreiber et al., 2001; Speake et al., 2002).

While the relationship between fatty acids and TH metabolism in brain development and function clearly needs further study, it seems probable that the abundant fatty acids apparent in the diet of *H. habilis* (and also later, as discussed below, in *H. sapiens*) may have evolutionary significance. When faced with reduced amounts of exogenous THs, abundant fatty acids in the diet may have optimized available THs and buffered the selection

processes that might otherwise have precipitated more profound and dramatic changes.

4. *Homo erectus:* beyond Africa

The next major morphological shift in the hominid lineage is evident in *Homo erectus*. *H. erectus* was definitely more human-like in proportions, with a significantly larger body and brain than its predecessore, a barrel-shaped ribcage, smaller teeth and a gracile mandible (Aiello and Wheeler, 1995; Fleagle, 1999). Tools and associated animal bones unequivocally establish this hominid as a consumer of a wide range of medium- and large-sized terrestrial mammals that inhabited open savannah-type environments. Although some of the larger prey species, such as elephants, may still have been scavenged, smaller taxa were undoubtedly hunted. As primary predators of such prey, thyroid glands and other organ tissues would have been available to these hominids. As a consequence, total exogenous THs consumed by *H. erectus* would have increased again over levels consumed by *H. habilis*. As for incipient Australopithecines, individuals with particular TR phenotypes would have retained reproductive function better under circumstances of increased consumption of exogenous thyroid hormones than others.

As THs so strongly control developmental programs, shifts in TH rhythms are probably responsible for the morphological changes to brain size and body proportions observed in *H. erectus*. The increased brain function and manual dexterity that must have accompanied these heterochronic morphological changes (McKinney, 2002; Parker, 2002) would have conferred distinct survival advantages. Once present in the founder population, traits such as larger size and a more complex brain could increase in frequency via selection for the developmental programs underlying those TH rhythms.

H. erectus survived as a species for well over 1 000 000 years and was the first hominid to move beyond Africa. There have been *H. erectus* fossils found in both Southeast Asia (Indonesia) and western Asia (Georgia) that date to approximately the same time as these fossil forms are found in Africa (Fig. 4) at ca. 1.8 mya. Lack of a continued presence in western Asia suggests this area was a transition zone for a single out-of-Africa emigration by *H. erectus*. There is no doubt that *H. erectus* lived as successfully in Asian habitats as in African ones (Fleagle, 1999). The early presence of *H. erectus* in Indonesia, combined with strong evidence of a continuous isolated existence in Asia (the lack of an Asian Acheulean tool tradition, for example, suggests this technology developed later in Africa alone), has caused some physical anthropologists to refer to the African form as *H. ergaster* and the Asian form as *H. erectus* (the Asian fossils, discovered first, have supremacy in taxonomic nomenclature if these are considered separate species).

In biological terms, however, this situation almost certainly qualifies for the application of subspecific rather than specific taxonomic distinctions, indicating geographically isolated populations of the same species (thus *H. erectus erectus* for the Asian form and *H. erectus ergaster* for the African). I refer to both as *H. erectus*, since subspecies are considered taxonomic conveniences rather than real evolutionary entities (Mayr, 1982; Park, 1999). The very recent report of fossils with '*H. erectus erectus*' morphology in Ethiopia dated at ca. 1 mya—a form not found in Africa before—seems to confirm this single species interpretation (Asfaw et al., 2002). Nevertheless, it is critical to acknowledge that a hominid of essentially identical form (that originated in Africa) came to live in both Asia and Africa, and that hominid species appears to be the direct ancestor of the *H. sapiens* lineage.

5. *Homo heidelbergensis* and *Homo neanderthalensis*

The speciation event that occurred next in the hominid lineage was the emergence of *Homo heidelbergensis*. An even larger-bodied, large-brained hominid, this species appears in the fossil record of Africa, Europe and Asia just after a major global climatic change that occurred approximately 1 mya (Stringer, 2002). There is little doubt that this speciation event occurred as a divergence from the *H. erectus* line, but what this species should be called is still hotly debated (Minugh-Purvis, 1996). While many regard the species that diverged from *H. erectus* as distinct from our own (*H. heidelbergensis*), others consider this the first appearance of *Homo sapiens*, albeit an 'archaic' form (Gamble, 1993; Relethford, 1997). Proponents of the term 'archaic *H. sapiens*' (an unorthodox category not used for other mam-

malian taxa) also lump the relatively distinctive Neanderthal and an earlier, possibly ancestral form into this group. I treat them separately here for reasons that will become apparent.

Early *H. heidelbergensis* fossils are relatively abundant, known from both Africa (Ethiopia at ca. 600 000 ya; Zambia at ca. 400 000 ya) and Europe (Germany and England at ca. 320 000–500 000 ya; Spain, France and Hungary at ca. 250 000–300 000 ya) (Park, 1999; Tattersall and Schwartz, 2001). *H. heidelbergensis* is considered by Stringer (2002) and others to be the common ancestor of *H. neanderthalensis* in Eurasia, and of *H. sapiens* in Africa. The systematic position of so-called *Homo antecessor*, a fossil hominid with a peculiar mix of both modern and primitive features found at ca. 780 000 ya in northern Spain (Arsuaga, 2000), is still heavily disputed (Stringer, 2002) and I have simply left them out of this discussion.

The morphological changes that characterize *H. heidelbergensis* are associated with the onset of cooler and drier Pleistocene environments, a time when many habitats experienced major faunal turnover. Such changes seem to have required hominids to intensify hunting over scavenging activities, perhaps due to increased competition from other carnivores for available carcasses. Such a dietary shift (more organ tissues) would have increased the relative proportions of exogenous THs, providing selection pressure favoring individuals with TR phenotypes that could handle higher levels of exogenous THs without disruption of reproductive function. At the same time, cooler temperatures would have supplied selection pressure to eliminate especially cold-sensitive TR phenotypes from the population.

As the Pleistocene epoch intensified, further adaptations became necessary in the northern extremes of *H. heidelbergensis* territory. Incipient Neanderthals were *H. heidelbergensis* colonizers of the Arctic steppe and tundra habitats that dominated the climate of Western Eurasia. These colonizers faced even cooler temperatures than *H. heidelbergensis* had before them, in addition to the increased food limitations and variability imposed by such extreme conditions. The oldest fossils of *H. neanderthalensis* come from Germany (dated at ca. 225 000 ya) and the total range of this species appears never to have expanded beyond Europe and western Asia (Stringer and Gamble, 1993; Mellars, 1996; Wolpoff, 1999; Manzi et al., 2000; Tattersall and Schwartz, 2001).

Neanderthal ancestors who chose to colonize the harsh and ever-shifting Pleistocene steppe environments must have consisted of a small group of individuals who were physiologically tolerant of the relatively severe climatic conditions of that new environment: they possessed one of several naturally-occurring physiological variants that existed naturally within the ancestral *H. heidelbergensis* population. Since TH metabolism is the body's mechanism for adjusting individuals to cold, distinct TR phenotypes within the ancestral Neanderthal population would have given some individuals higher tolerance to the physiological stress of reduced temperatures than others. Temperatures need not have been frigidly arctic, just significantly colder than the habitats of their ancestors; negatively selected individuals did not necessarily die, they just produced few or no surviving offspring.

The reduced variation of TH phenotypes manifested in this small population of incipient Neanderthals would have established a distinctive hormonal pattern in their descendants that differed significantly from the common ancestral pattern. Compounding the selection pressure of cold temperatures would have been the extremely high proportion of the diet necessarily composed of raw meat and organ tissue, simply because far fewer plant products would have been available. Raw meat still appears to have been the dominant dietary component of all hominids at this time, with little compelling evidence for the use of fire for cooking rather than for heat and light (Fleagle, 1999; Arsuaga, 2000). *H. heidelbergensis* in more temperate climates may have consumed moderate quantities of plant material (either year round or seasonally), but it is doubtful that this option was available to Neanderthals.

Since Neanderthals are in many ways merely a more cold-tolerant form of *H. heidelbergensis*, we would expect to find much smaller morphological and developmental differences between the two than between Neanderthals and modern humans. For example, analysis of mineral levels in *H. neanderthalensis* bones attest to the fact that a very high proportion of the Neanderthal diet was composed of red meat (Mellars, 1996; Balter et al., 2001). Such a diet would have provided a much greater amount of exogenous THs, especially if thyroid glands were occasionally eaten, than exists in the diets of modern *H. sapiens*. If Neanderthals consumed significantly higher amounts of

exogenous TH than modern humans, they must have had a faster turnover rate for THs than the modern rate of 6.8 days (Kaptein et al. 1994). A faster turnover rate for THs suggests that a distinctly different TR must have existed for *H. neanderthalensis*.

More direct evidence of distinctive Neanderthal TRs comes from new chronological aging techniques that measure incremental growth lines in tooth enamel (perikymata). Aging studies on selected fossils suggest that *H. neanderthalensis* had faster post-natal growth rates than do modern humans (Stringer and Gamble, 1993; Ramirez Rozzi, 2002) and analysis of skull shapes during ontogeny reveal that craniofacial growth patterns are distinct (Ponce de León and Zollilofer, 2001; Minugh-Purvis, 2002). A recent study by Williams et al. (2002) compared modern human and Neanderthal craniofacial growth patterns to another closely related species pair—the common chimpanzee, *P. troglodytes*, and the pygmy chimpanzee or bonobo, *P. panisus*. Their study confirms a faster post-natal growth rate for Neanderthals relative to modern humans from the early post-natal period onwards. More significantly, these differences are at least as great as, or greater than, those manifested by different species of *Pan*. Such evidence that different post-natal growth rates for *H. neanderthalensis* existed is the most reliable indicator that this hominid possessed its own distinct TR.

The evidence that Neanderthals possessed a distinctive TR supports the view that they represent a distinct species of *Homo* rather than a subspecies or 'archaic' form of *H. sapiens* (Ponce de León and Zollilofer, 2001; Williams et al., 2002), a position supported by recent genetic studies. Analysis of Neanderthal mtDNA sequences strongly suggests that they were a genetically distinct lineage that had been reproductively isolated for a considerable length of time (Lindahl, 1997; Krings et al., 1997, 2000; Ward and Stringer, 1997; Ovchinnikov et al., 2000), although some critics are still not convinced (Relethford, 2001). The combined evidence, however, presents a very strong case for making a taxonomic distinction between Neanderthals and modern humans (Tattersall and Schwartz, 2001; Stringer, 2002).

Reluctance to classify Neanderthals as a distinct species is due in part to a misguided belief that this would necessarily rule out interbreeding between the two when declining numbers of *H. neanderthalensis* populations met increasing numbers of early *H. sapiens* at the end of the Pleistocene era (Gamble, 1993; Trinkaus and Shipman, 1993; Wolpoff, 1999). However, recent molecular data confirm that hybridization between closely related species is much more common than previously thought. Hybridization has been shown to be neither rare nor 'unnatural' in closely related animals (Arnold, 1997; Crockford, 2000b; Schillaci and Froehlich, 2001; Grant and Grant, 2002; Levin, 2002) and hominids were not likely an exception. A few Neanderthals could very easily have been assimilated into populations of early *H. sapiens*. Evidence of just such a scenario is provided by a putative modern-Neanderthal hybrid specimen recently found in Portugal (Duarte et al., 1999), although Dobson and Geelhoed (2001) suggest another interpretation for these remains.

An additional argument against classifying classic *H. neanderthalensis* fossils as representatives of a distinct species lies in their morphological similarity to some early *H. sapiens* fossils (Hawks and Wolpoff, 2001; Wolpoff et al., 2001). However, a cold-tolerant (incipient Neanderthal) phenotype did not cease to exist in *H. heidelbergensis* ancestral populations simply because some individuals with those qualities chose to leave the group—some cold-tolerant individuals would have stayed, contributing the genes for this phenotype to future generations. Thus, early *H. sapiens* would also have had a relatively cold-tolerant phenotype as one variant among many, a range of variation necessary for them to become a successful, globally distributed species.

6. Anatomically modern humans, *Homo sapiens*

The last significant change in the *Homo* lineage is the emergence of so-called anatomically modern humans, *H. sapiens*. Fossils from Africa attributed to this species have been dated at almost 150 000 ya, while some found in Israel are closer to 100 000 ya (Stringer, 2002). However, abundant specimens are not found until approximately 36 000 ya in Europe and ca. 30 000 ya in Asia (Park, 1999: 264), with a few more from the 40 000–50 000-year range. The biological changes here are slight: the teeth are smaller, heavy brow ridges are essentially gone, and the skeleton overall is less robust. An almost vertical forehead in *H. sapiens* gives the face a flatter profile with a protruding chin. Culturally, there are the begin-

nings of truly 'modern' human behavior: more complex and varied tool types, highly decorated art objects, musical instruments and painting, and most important to this story, the regular use of fire for cooking (Fleagle, 1999: 537).

Definitive evidence for the frequent use of fire for cooking is available for only the most recent finds (ca. 30 000 ya) and is perhaps not coincidentally associated with the most dramatic of the cultural artifacts, such as Aurignacian tools and elaborate cave paintings. I suggest that whenever (and wherever) cultural stimuli and technical skills led to the regular practice of cooking meat, it precipitated the physiological and morphological changes in descendant populations that we recognize as *H. sapiens*, because it reduced the amount of exogenous THs in meat. Although data on the response of THs to heat of various temperatures are slim, it is known that the heat required for drying thyroid tissue (ca. 100–120 °C) is insufficient to affect either T_3 or T_4 (Hadley, 1984). Much higher temperatures, especially those required for thorough cooking via simple, direct methods such as spit-roasting (ca. 400–600 °C), probably cause almost complete degradation of T_3 (since it is a less stable molecule) and perhaps modest degradation of T_4. Thus, the regular practice of cooking meat rather than eating it raw would have decreased the exogenous THs (but especially T_3) in the diet of many widespread populations of incipient *H. sapiens*. As in previous declines of THs, biological consequences ensued.

A profound decrease in exogenous T_3 seems to have precipitated selection pressure on the thyroid glands of incipient *H. sapiens* to provide virtually all of the T_3 required for normal physiological function. Individuals whose thyroid metabolism could not function optimally without some influx of exogenous T_3 would gradually have become minor constituents of the population because of the tight link of THs to reproductive function. Only those individuals whose thyroid glands could readily supply all of the T_3 needed would produce abundant offspring.

Regardless of when and where the cooking innovation occurred, rapid and widespread morphological and behavioral changes probably followed. The fact that many morphological traits are linked to brain development through TH-controlled embryonic and postnatal growth programs means that the survival advantages conveyed by a more complex brain to any early *H. sapiens* could easily have generated descendants with smaller teeth, gracile muscular and a shorter gut as an indirect consequence (cf. Aiello and Wheeler, 1995). This suggests that changes in the skeletal morphology of *H. sapiens* documented in the archaeological record are real and significant manifestations of selection for increased brain complexity. Neurological pathways were added that had not been present before (McKinney, 2002; Parker, 2002), circuits that made it possible for complex language and culture to develop.

There really is no other plausible external explanation except the regular cooking of meat for the sudden explosion of cultural behavior accompanied by marked biological changes in early *H. sapiens*—no other major changes occurred. I suggest that the appearance of a few anatomically modern humans at ca. 100 000 ya or earlier may reflect a local reliance on fish and shellfish, remains of which have been found associated with some of these older sites (e.g. Brooks et al., 1995). Such diets would have been rich in essential fatty acids but not exogenous THs, since fish are unlikely to have been eaten whole. Such local dietary adaptations could have generated anatomically modern features within relatively small and isolated populations, but perhaps could not precipitate the dramatic burst of cultural innovations we see later simply because there were not as many individuals involved.

Alternatively, the innovation of using fire for cooking could also have arisen independently and sporadically at first, with local morphological effects as described above. The full range of cultural and behavioral effects, however, may have required the involvement of many more individuals, a situation that did not arise until almost 40 000 ya. Eventually, enough incipient *H. sapiens* were using fire to cook their meat that the biological effects became widespread, finally generating enough brain-enhanced human minds interacting that it fueled an associated explosion of cultural innovation.

If the regular use of fire for cooking was indeed the stimulus that precipitated the biological and cultural changes associated with fully modern *H. sapiens* in Africa, it is entirely possible that *H. sapiens* could have arisen independently in Asia from local populations of *H. heidelbergensis*. Indeed, Asian forms of *H. heidelbergensis* could themselves have descended from local Asian *H. erectus* populations just as easily (if not more so)

as they could have migrated in from Africa (Fig. 4). Geographically isolated subspecies of *H. erectus* lived in both continents and would have responded in similar fashion to similar environmental pressures. I suggest the morphological responses of both populations could have been so similar that we would be hard-pressed to tell fossils of them apart.

Genetic support for the controversial palaeontological evidence that *H. sapiens* may have had polyphyletic origins (Stringer, 2002) seemed to come from a recent study of mtDNA extracted from ancient Australians (Adcock et al., 2001a; Relethford, 2001). However, criticisms of these data have been so diverse (Adcock et al., 2001b; Colgan, 2001; Groves, 2001; Trueman, 2001; Stringer, 2002) that it is virtually impossible to determine if indeed the data provide evidence, independent of morphological criteria, that multiple origins actually occurred. More ancient genetic data and well-dated fossils are still needed to resolve the issue, although even if *H. heidelbergensis* and *H. sapiens* did arise independently in Asia, their populations could still have been culturally and genetically swamped by later-arriving representatives of African origin.

7. Recent dietary adaptations

Evolution is a continuous process and small, additional changes in human developmental programs (as reflected in skeletal configuration and physiology) are still underway, some of which began to show themselves only after the agricultural revolution. Generally speaking, before the practice of deliberate cultivation of cereal grains began, most human populations were hunter/gatherers who depended on meat and fat sources for most of their calories, supplemented by whatever edible plants, nuts and seeds were available. Only when cereal grains and starchy root crops could be produced in dependable quantities and stored for future use did carbohydrates come to represent a dominant staple of the hominid diet. Human culture and social organization changed dramatically in every population that experienced this mastery of carbohydrates (Diamond, 1999, 2002). Although local reliance on collected wild progenitors preceded organized cultivation by as much as 10 000 years, crop domestication occurred quite late in our long evolutionary history. Evidence of domestic crops are found in the Middle East and China at approximately 8000–9000 ya and much later, at ca. 5000 ya, in Mesoamerica (Harris, 1996).

The relevance of this revolution to my argument is that an abundance of staple cereal or starchy root crops in the diet reduced further still whatever component of exogenous T_4 that remained. Of course, some cultures did not adopt agriculture at all (as on the northwest coast of North America or in the Arctic). However, in regions where agriculture has its longest history, such as the Middle East and Asia, diets increasingly came to be dominated by carbohydrates rather than meat as early as 20 000 ya (Schoeninger, 1982). As the relative proportion of meat consumption dropped, TH physiology would have had to shift to accommodate the loss of virtually all dietary T_4. Due to the tight association of THs with reproductive function, those individuals whose TR phenotypes generated optimal amounts of THs would have been more reproductively successful than those who needed significant supplemental T_4 from dietary sources.

Although the morphological changes associated with these adaptations would have been small in comparison to previous evolutionary shifts, such changes are nevertheless evident in skeletal samples and prove to be heterochronic in nature. Minugh-Purvis (2002, p. 496), for example, found that some heterochronic changes in the posterior neurocranium have appeared since the emergence of *H. sapiens* (i.e. between samples ca. 30 000 years old and those less than 6000 years old), and states that "early Upper Paleolithic Europeans, usually regarded as early modern humans based on both behavioral and morphological criteria, were not fully modern in their growth patterning." Thus, the tight correlation between THs, growth and reproductive function was instrumental in continuing to adapt late modern humans to dietary changes that were largely independent of changing environmental conditions.

8. Health implications

Modern humans, with their very slow turnover rate for T_4, now appear to be well adapted to a diet lacking any appreciable amounts of THs. However, our tolerance to fluctuations in TH levels is now very low indeed and either too much (hyperthyroidism) or too little (hypothyroidism)

make us decidedly ill (Braverman and Utiger, 1991).

How many people are affect by TH imbalances? Hollowell et al. (2002) tested over 17 000 Americans for serum TSH, T_4 and thyroid antibody levels. Extrapolation of their survey results predicted that within the US alone more than 8 000 000 people unknowingly have laboratory evidence of hypothyroidism and that approximately 700 000 people unknowingly have hyperthyroidism. In addition, the survey suggested that a significant number of people (more than 30%) who are already taking medication for thyroid imbalances are not being treated effectively. As high as these numbers seem, they are almost certainly too low, since they are based on using a very wide range of serum TSH concentration readings (0.5–4.5 MU/l) as indicators of 'normal' function (values exceeding high end values indicate hypothyroidism).

Many doctors now consider that TSH readings above 2.0 MU/l (especially when accompanied by symptoms) are valid indicators of hypothyroidism or incipient hypothyroidism (Arem and Escalante, 1996; Arem, 1999; Weetman, 1997; Hanna et al., 1999; O'Reilly, 2000). As Weetman (1997, p. 1175) points out in his discussion of a thyroid function survey carried out in Britain that retested subjects after 20 years (Vanderpump et al., 1995), "the simplest explanation is that thyroid disease is so common that many people predisposed to thyroid failure are included in a laboratory's reference population." A recent Danish study on healthy males tested thyroid function in 16 individuals repeatedly over a 12-month period and found a wide range of variation between individuals for all values of serum T_4, T_3, free T_4 and TSH (Andersen et al., 2002).

Similarly, in veterinary medicine, the use of the TSH test for diagnosing hypothyroidism in dogs is also disputed (Greene, 1997). In dogs, the 'normal' range for TSH concentrations established by laboratories came from testing a reference population that lumped many breeds together for a general dog value. It is now acknowledged that each breed probably has a different value for 'normal' (Ferguson, 1997), although individual variation in values and the pulsatile nature of TH secretion are still not acknowledged. Consequently, in both human and canine cases, when laboratory tests alone are used to guide the clinician, diagnosis of hypothyroidism is haphazard and treatment often ineffective in resolving all symptoms of the disorder.

Although we do not have data from 10 000 ya to know if the prevalence of hypothyroidism is a new phenomenon for humans, there are nevertheless millions of people worldwide currently afflicted by symptoms of hypothyroidism. Doctors can be confused by the wide-ranging consequences of hypothyroidism, since long lists of symptoms are often presented in idiosyncratic arrays: depression; lethargy; insomnia; weight gain; constipation; impaired concentration; muscle and joint pain; cold intolerance; dry skin; heavy menstruation; hair loss; high cholesterol (and associated heart problems); infertility; miscarriage; birth defects, etc. (Weetman, 1997; Arem, 1999; Cudd et al., 2002). Hypothyroidism has also been strongly linked to hyperactivity disorders in children (Hauser et al., 1997; Hauser and Rovet, 1998) and to postpartum depression (Othman et al., 1990). People are not dying of these disorders, but they are often rendered under-productive or even non-productive, and doctors are often not able to help.

Even when a diagnosis of hypothyroidism has been made, treatment is not always successful at relieving patients' symptoms. Some of this failure, as suggested above, may be due to a reliance on TSH values alone to indicate when 'normal' levels of T_4 have been restored. This approach may not supply enough T_4 for some individual needs. Supplementation with synthetic T_4 has been the standard treatment for human hypothyroidism for at least 30 years (Arem, 1999), a strategy that assumes the body's natural conversion mechanism of T_4 will yield all of the T_3 required. Surprisingly, supplementation with both T_3 and T_4 has been shown to resolve many symptoms that remain when T_4 alone is given (Arem, 1999; Bunevicius et al., 1999), suggesting that this conversion mechanism can be impaired in some patients. However, it has also been shown that some tissues, particularly the brain, have a higher requirement for T_3 than others (Bunevicius et al., 1999). Clearly, the mechanism responsible for regulating optimal T_3 levels in various tissues (via thyroid gland secretion and T_4 conversion) is more complex and variable than previously thought.

Untreated maternal hypothyroidism, as discussed previously, can cause significant birth defects in infants, particularly in brain growth and development (Braverman and Utiger, 1991; Geelhoed, 1999). Not surprisingly, disruption of mater-

nal TH production may be the mechanism underlying the physical and brain development anomalies known as fetal alcohol syndrome. Alcohol appears to suppress thyroid levels in both the mother and fetus, resulting in birth defects that are very similar to infants born to hypothyroid mothers (Cudd et al., 2002). Some common pharmacological agents (including aspirin, general anesthesia, Phenobarbital, etc.) are also known to suppress or disrupt thyroid function (Ferguson, 1997; Hanna et al., 1999).

Naturally occurring biochemical compounds can also affect thyroid function. Goiterogens present in some vegetables, such as species of Brassicae (the cabbage family), sorghum, sweet potatoes, maize, almonds and cassava, are very similar in molecular structure to THs. Goiterogens attach themselves to TH receptors, and in large enough quantities can produce a hypothyroid state by competitive exclusion (Hadley, 1984). For example, the extremely high incidence of goiter caused by insufficient dietary iodine in some African regions is exacerbated by a reliance on cassava as the primary source of calories (Geelhoed, 1999). Synthetic chemicals that are comparable in molecular structure to THs, such as dioxins and polychlorinated biphenyls (PCBs), may similarly affect normal fetal and early postnatal brain development because they either reduce or mimic TH actions (Porterfield, 1994; Hauser et al., 1998).

Apart from such biochemical hazards, stress and age are two inescapable factors known to inhibit TH production (Hadley, 1984). Stress can be emotional or physical and may have temporary or permanent effects on human health. Stress-induced reductions in TH production can precipitate coordinated reductions in growth hormones, cortisol and gonadal hormones, resulting in an often confusing array of physical and psychological symptoms (Arem and Escalante, 1996; Arem, 1999). Blackburn-Munro and Blackburn-Munro (2001), for example, suggest that the depression often associated with chronic pain might be the same physiological manifestation as clinical depression associated with other kinds of inescapable chronic stresses.

Depression is a common symptom of both hypothyroidism and chronic stress, although it is most often treated as a distinct ailment. Antidepressant drugs such as Prozac or Zoloft ('selective serotonin reuptake inhibitors' or SSRIs), are widely used in treating depression (Weissman, 2001). However, antidepressants cannot 'fix' depression caused or exacerbated by hypothyroidism or chronic stress, and such drugs often have serious side effects of their own (Jenkins, 2001). Unsuspected hypothyroidism in many patients (either primary or caused by chronic stress) may account for the enormous individual variation in success rates in treating depression with drugs (Arem, 1999; Doyle Driedger, 2001).

In addition to reductions associated with stress, TH levels also decline with age. By age 85, basic metabolic rate has dropped to 52% of what it was at 3 years of age (Arem, 1999: 57). Age-related disorders, such as poor temperature regulation, insomnia, decreased mental activity, dry skin, constipation, depression, muscle weakness or stiffness, etc., may ultimately be caused by declining TH levels or disruption of TH rhythms. As we expect ourselves and others to remain active and useful well into old age, finding effective diagnosis and treatment regimes for thyroid disorders takes on increasing urgency (Arem, 1999; Dunn, 2002).

It should be clear from this discussion that doctors currently do not have a very precise understanding of what constitutes normal TH function and its range of variability, nor of the full consequences when disruption of the system occurs. Therefore, it is not surprising to find that none of the current research and discussion regarding TH disorders in human or veterinary medicine acknowledges the pulsatile nature of TH production. Single blood tests sample the rhythm at one point in a continuum: how can doctors possibly interpret what that value really means?

TH rhythms may be significantly disrupted in afflicted individuals even if TSH values never fall outside normal ranges or critical threshold peaks of TH concentration may not be attained at appropriate times. While rhythm disruption of insulin has been identified in diabetes (Butler, 2000) and for disorders of several other hormones that exhibit pulsatile action (Brabant and Prank, 2000; Schaefer, 2000; Velduis, 2000), no one seems to have considering testing for rhythm disruption of THs. Research into individual differences that exist at the receptor level are also clearly needed, since there is evidence that some people have, or can develop, a resistance to TH at the tissue level (Refetoff et al., 1993), as can occur with insulin (Martin et al., 1992).

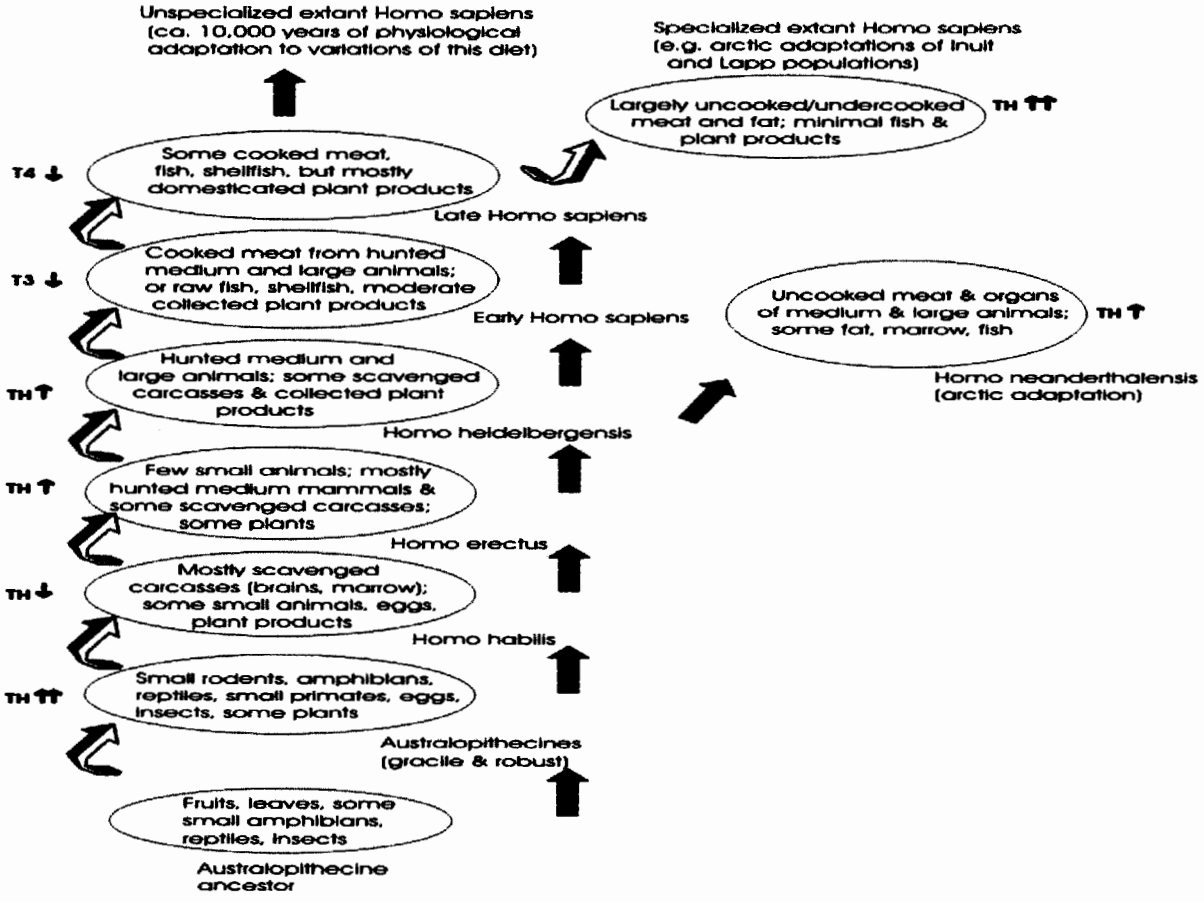

Fig. 5. Summary of the dietary changes proposed during hominid evolution and the changes in exogenous THs they represent.

9. Summary and conclusions

The concept summarized here proposes that genetically controlled, species-specific (but individually variable) rhythms of THs constitute the central control mechanism for speciation and adaptation changes in the evolution of all vertebrates, including humans. It explains how selection for particular, naturally occurring variants of TH production rhythms in hominid ancestors, when isolated within small inter-breeding populations of colonizers, could quickly generate new hominid species with distinctive skeletal, physiological and behavioral characteristics. Dietary changes associated with colonization of new habitats, or adaptation to changes in existing habitats, involved shifts back and forth in relative amounts of exogenous T_3 and T_4, and these appear to have driven all but the most recent biological modifications, which were entirely diet-driven (Fig. 5).

The morphological characteristics that emerged over the course of hominid evolution (including increased linear stature, increased cranial capacity and complexity pelvic and femoral changes, shortening of the gut, slowing of tooth eruption) are virtually all heterochronic in nature and are thus traits potentially produced by the effects of THs on growth and development. Due to the fact that THs ultimately control reproductive function, hair growth and color, and the stress response, dietary shifts that impacted developmental programs even slightly would have affected many seemingly unrelated traits as a consequence. For example, the two distinctive features of modern humans that have been the hardest to explain as selected traits–hairlessness and fat accumulation–may simply

have been inevitable consequences of the heterochronic changes that took place in the hominid lineage. In addition, maternally produced and consumed THs, through their effects on fetal growth and development, would have been a significant non-genetic modifier of an individual's phenotype. Consequently, maternally controlled effects on embryonic development might have been somewhat different than later effects on postnatal growth and brain development governed by the individual's own genes.

Fluctuating diets may have been instrumental in maintaining a large pool of individual TH rhythm variation in the hominid lineage, variation that was ultimately essential to the success of our species. More than once over the course of hominid evolution, when the amounts of exogenous THs were low (particularly for *H. habilis* and *H. sapiens*), the consumption of foods rich in essential fatty acids (such as meat, marrow, brains, fish and eggs) may have buffered the natural selective forces that might otherwise have generated more pronounced changes. An even more direct relationship may exist: Horrobin (1997; Horrobin, 2001) has suggested quite convincingly that the apparently simultaneous appearance of schizophrenia (which recent evidence suggests is at least partly due to a genetic deficiency in EPA production or utilization) and the emergence of modern human culture may be of evolutionary significance (see also Horrobin and Bennett, 1999). He points out the strong familial inheritance patterns, suggestive of a genetic correlation, that connect certain mental disorders (including full-blown schizophrenia, schizo-typal behaviour, manic-depression/bipolar disorder and dyslexia) and especially creative or productive traits (including scientific inventiveness, academic acumen, religious fervor, artistic and musical talent). Horrobin suggests that essential fatty acids in the diet of hominid ancestors may have been instrumental in shaping our brain into its essential "human" qualities, a proposition perhaps deserving serious investigation given the critical role for THs in heterochronic change described here and the associated correlation between THs and essential fatty acids in governing brain developmental and function.

The innovation of using fire to cook meat appears to be the stimulus that finally pushed our ancestors over an important evolutionary line. Since changing the nutritional constituents of an established diet simply by preparing it differently is something only hominids could have done, it is using fire for cooking that in the end made us a truly unique species in evolutionary terms. It is also true, however, that as soon as hominids had even the crudest of tools more than 2 mya, they had more choices in their food sources than other primates, and those choices helped set the path of their biological evolution.

This new paradigm provides the first truly plausible biological explanation for a number of aspects of hominid history: the convergent evolution of bipedal morphology in non-hominid apes; the different degrees of sexual dimorphism in distinct hominid species; the coordinated changes in morphology, brain function and gut length over time that have been so confounding; the very real possibility of an independent evolution of *H. heidelbergensis* and *H. sapiens* from genetically distinct geographic subspecies of *Homo erectus*; and regional adaptation of geographically distinct populations.

Although we will never know for sure if the particular scenarios proposed here actually produced past hominid changes in the ways I have suggested, we can test the underlying biological assumptions using animal model systems because they should work similarly for all vertebrates. However, there are compelling reasons, apart from understanding our history, for needing to know if this theory presents a useful model: if my theory is correct, there are enormous implications for human healthcare. We are in desperate need of research that documents the range of normal human TH rhythms and studies which demonstrate how disruption of these rhythms (due to stress, disease, drugs and biochemical pollutants) affect day-to-day health. Individual variation in TH rhythms (TR phenotypes) appear to be essential to the ability of hominids to adapt to changing conditions of life: this is as true today for modern humans as it was 5 000 000 years ago for our ancestors.

Acknowledgement

Everlasting gratitude to the memory of Editor Peter Hochachka for offering the challenge, and the opportunity of presenting this paper.

References

Adcock, G.J., Dennis, E.S., Easteal, S., et al., 2001. Mitochondrial DNA sequences in ancient Australians: implications for modern human origins. Proc. Natl. Acad. Sci. USA 98, 537–542.

Adcock, G.J., Dennis, E.S., Easteal, S., et al., 2001. Lake Mungo 3: a response to recent critiques. Arch. Oceania 36, 170–174.

Aiello, L.C., Wheeler, P., 1995. The expensive tissue hypothesis: the brain and digestive system in human and primate evolution. Curr. Anthropol. 36, 199–221.

Andersen, S., Pedersen, K.M., Bruun, N.H., Laurberg, P., 2002. Narrow individual variations in serum T_4 and T_3 in normal subjects: a clue to the understanding of subclinical thyroid disease. J. Clin. Endocrinol. Metab. 87, 1068–1072.

Arem, R., Escalante, D., 1996. Subclinical hypothyroidism: epidemiology, diagnosis, and significance. Adv. Intern. Med. 41, 213–250.

Arem, R.., 1999. The Thyroid Solution. Ballantine Books, New York.

Arnason, U., Bodin, K., Gullberg, A., Ledje, C., Mouchaty, S., 1995. A molecular view of pinniped relationships with particular emphasis on the true seals. J. Mol. Evol. 40, 78–85.

Arnold, M.L., 1997. Natural Hybridization and Evolution. Oxford University Press, Oxford.

Arsuaga, J.L., 2000. The first Europeans: Spanish caves paint a new picture of evolution on the continent. Discover. Archaeol. Nov/Dec, 48–65.

Asfaw, B., Gilbert, W.H., Beyene, Y., et al., 2002. Pleistocene hominids from Bouri, Middle Awash, Ethiopia integrate Homo erectus. Nature 416, 317–320.

Balter, , Person, A., Labourdette, N., Drucker, D., Renard, M., Vandermeersch, B., 2001. Les Néandertaliens étaient-ils essentiellement carnivores? Résultats préliminaires sur les teneurs en Sr et en Ba de la paléobiocénose mammalienne de Saint-Cesaire. C. R. Acad. Sci. Paris Earth Planet. Sci. 332, 59–65.

Barres, B.A., Lazar, M.A., Raff, M.C., 1994. A novel role for thyroid hormone, glucocorticoids and retinoic acid in timing oligodendrocyte development. Development 120, 1097–1108.

Belyaev, D.K., 1979. Destabilizing selection as a factor in domestication. J. Hered. 70, 301–308.

Berge, C., 2002. Peramorphic processes in the evolution of the hominid pelvis and femur. In: Minugh-Purvis, N., McNamara, K. (Eds.), Human Evolution through Developmental Change. Johns Hopkins University Press, Baltimore, pp. 381–404.

Bitman, J., Stanislaw, K., Wood, D.L., Lefcourt, A.M., 1994. Circadian and ultradian rhythms of plasma thyroid hormone concentrations in lactating dairy cows. Am. J. Physiol. 266, R1797–R1803.

Blackburn-Munro, G., Blackburn-Munro, R.E., 2001. Chronic pain, chronic stress and depression: coincidence or consequence? J. Neuroendocrinol. 13, 1009–1023.

Braverman, L.D., Utiger, R.D., 1991. Werner and Ingbar's The Thyroid. Lippincott, Philadelphia.

Brent, G.A., 2000. Tissue-specific actions of thyroid hormone: insights from animal models. Rev. Endocr. Metab. Disord. 1, 27–33.

Brent, G.A., Moore, D.D., Larsen, P.R., 1991. Thyroid hormone regulation of gene expression. Annu. Rev. Physiol. 53, 17–35.

Brooks, A.S., Cramer, J.S., Franklin, A., et al., 1995. Dating and context of three middle stone age sites with bone points in the upper Semliki valley, Zaire. Science 268, 548–553.

Bunevicius, R., Kazanavicius, G., Zalinkevicius, R., Prange Jr, A.J., 1999. Effects of thyroxine as compared with thyroxine plus triiodothyronine in patients with hypothyroidism. N. Engl. J. Med. 340, 424–429.

Brabant, G., Prank, K., 2000. Prediction and significance of the temporal pattern of hormone secretion in disease states. In: Chadwick, D.J., Goode, J.A. (Eds.), Mechanisms and Biological Significance of Pulsatile Hormone Secretion. J. Wiley and Sons, Chichester, pp. 105–118.

Butler, P., 2000. Pulsatile insulin secretion. In: Chadwick, D.J., Goode, J.A. (Eds.), Mechanisms and Biological Significance of Pulsatile Hormone Secretion. J. Wiley and Sons, Chichester, pp. 190–205.

Chadwick, D.J., Goode, J.A., 2000. Mechanisms and Biological Significance of Pulsatile Hormone Secretion. J. Wiley and Sons, Chichester.

Colgan, D.J., 2001. Commentary on G.J. Adcock, et al., 2001 Mitochondrial DNA sequences in ancient Australians: implications for modern human origins. Arch. Oceania 36, 168–169.

Coppinger, R., Schneider, R., 1995. Evolution of working dogs. In: Serpell, J. (Ed.), The Domestic Dog: Its Evolution, Behaviour and Interactions with People. Cambridge University Press, Cambridge, UK, pp. 21–47.

Crockford, S.J., 2000a. Dog evolution: a role for thyroid hormone physiology in domestication changes. In: Crockford, S.J. (Ed.), Dogs Through Time: An Archaeological Perspective, British Archaeological Reports S889. Oxford, pp. 11–20.

Crockford, S.J., 2000b. A commentary on dog evolution: regional variation, breed development and hybridisation with wolves. In: Crockford, S.J. (Ed.), Dogs Through Time: An Archaeological Perspective, British Archaeological Reports S889. Oxford, pp. 295–312.

Crockford, S.J., 2002a. Animal domestication and heterochronic speciation: the role of thyroid hormone. In: Minugh-Purvis, N., McNamara, K. (Eds.), Human Evolution through Developmental Change. Johns Hopkins University Press, Baltimore, pp. 122–153.

Crockford, S.J., 2002b. Thyroid hormone in Neandertal evolution: a natural or a pathological role? Geog. Rev. 92, 73–88.

Cudd, T.A., Chen, W.-J.A., West, R.W., 2002. Fetal and maternal thyroid hormone responses to ethanol exposure during the third trimester equivalent of gestation in sheep. Alcohol Clin. Exp. Res. 26, 53–58.

Darwin, C., 1859. On the Origin of Species. J. Murray, London.

Darwin, C., 1868. The Variation of Animals and Plants Under Domestication. J. Murray, London.

Davilla, N., Shea, B.T., Omoto, K., Mercado, M., Misawa, S., Baumann, G., 2002. Growth hormone binding protein,

insulin-like growth factor-I and short stature in two pygmy populations from the Philippines. J. Pediatr. Endocrinol. Metab. 15, 269–276.

De Pablo, F., 1993. Introduction. In: Schreibman, M.P., Scanes, C.G., Pang, P.K.T. (Eds.), The Endocrinology of Growth, Development, and Metabolism of Vertebrates. Academic Press, New York, pp. 1–11.

Diamond, J., 1999. Guns, Germs and Steel: The Fates of Human Societies. W.W. Norton & Co, New York.

Diamond, J., 2002. Evolution, consequences and future of plant and animal domestication. Nature 418, 700–707.

Dobson, J.E., Geelhoed, G.W., 2001. The Châtelperronian/Aurignacian conundrum: one culture, multiple human morphologies? Curr. Anthropol. 42, 139–140.

Doyle Driedger, S., 2001. Overcoming depression: one woman's terrifying odyssey through a nightmare of despair. McClean's 114 (46), 34–38.

Duarte, C., Maurício, J., Pettitt, P.B., et al., 1999. The early Upper Paleolithic human skeleton from Abrigo do Lagar Velbo (Portugal) and modern human emergence in Iberia. Proc. Natl. Acad. Sci. USA 96, 7604–7609.

Dunn, J.T., 2002. Editorial: Guarding our nation's thyroid health. J. Clin. Endocrinol. Metab. 87, 486–488.

Ferguson, D.C., 1997. Euthyroid sick syndrome. Canine Pract. 22, 49–51.

Fleagle, J.G., 1999. Primate Adaptation and Evolution. 2nd ed.. Academic Press, San Diego.

Gagneux, P., Amess, B., Diaz, S., et al., 2001. Proteomic comparison of human and great ape blood plasma reveals conserved glycosylation and differences in thyroid hormone metabolism. Am. J. Phys. Anthropol. 115, 99–109.

Gamble, C., 1993. Timewalkers: The Prehistory of Global Colonization. Alan Sutton, Phoenix Mill, UK.

Gancedo, B., Alonso-Gomez, A.L., de Pedro, N., Delgado, M.J., Alonso-Bedate, M., 1997. Changes in thyroid hormone concentrations and total contents through ontogeny in three anuran species: evidence for daily cycles. Gen. Comp. Endocrinol. 107, 240–250.

Geelhoed, G.W., 1999. Metabolic maladaptation: individual and social consequences of medical intervention in correcting endemic hypothyroidism. Nutrition 15, 908–932.

German, R.Z., Stewart, S.A., 2002. Sexual dimorphism and ontogeny in primates. In: Minugh-Purvis, N., McNamara, K. (Eds.), Human Evolution through Developmental Change. Johns Hopkins University Press, Baltimore, pp. 207–222.

Gould, S.J., 1977. Ontogeny and Phylogeny. Harvard University Press, Cambridge.

Grant, P.R., Grant, B.R., 2002. Unpredictable evolution in a 30-year study of Darwin's finches. Science 296, 707–711.

Greene, R.T., 1997. Thyroid testing: the full circle. Canine Pract. 22, 10–11.

Groves, C., 2001. Lake Mungo 3 and his DNA. Arch. Oceania 36, 166–167.

Hadley, M.E., 1984. Endocrinology. Prentice-Hall Inc, New Jersey.

Haisenleder, D.J., Ortolano, G.A., Dalkin, A.C., Yasin, M., Marshall, J.C., 1992. Differential actions of thyrotropin (TSH)-releasing hormone pulses in the expression of prolactin and TSH subunit messenger ribonucleic acid in rat pituitary genes in vitro. Endocrinology 130, 2917–2923.

Hanna, F.W.F., Lazarus, J.H., Scanlon, M.F., 1999. Controversial aspects of thyroid disease. Br. Med. J. 319, 894–899.

Härlid, A., Arnason, U., 1999. Analysis of mitochondrial DNA nest ratite birds within the Neognathae: supporting a neotenous origin of ratite morphological characters. Proc. R. Soc. Lond. B. 266, 305–309.

Harris, D.R., 1996. The origins and spread of agriculture and pastoralism in Eurasia: an overview. In: Harris, D.R. (Ed.), The Origins and Spread of Agriculture and Pastoralism in Eurasia. Smithsonian Institution Press, Washington, DC.

Hauser, P., Rovet, J., 1998. Thyroid Diseases of Infancy and Childhood. American Psychiatric Press Inc, Washington, DC.

Hauser, P., Soler, R., Brucker-Davis, F., Weintrub, B.D., 1997. Thyroid hormones correlate with symptoms of hyperactivity but not inattention in attention deficit hyperactivity disorder. Int. J. Psychol. Neurol. Endocrinol. 22, 107–114.

Hauser, P., McMillin, J.M., Bhatara, V.S., 1998. Thyroid hormone disruption: dioxins linked to attention deficit, learning problems. Toxicol. Ind. Health 34, 85–101.

Hawks, J.D., Wolpoff, M.H., 2001. The accretion model of Neanderthal evolution. Evolution 55, 1474–1485.

Hayes, T.B., 1997. Hormonal mechanisms as potential constraints on evolution: examples from the Anura. Am. Zool. 37, 482–490.

Hayes, J.P., Jenkins, S.H., 1997. Individual variation in mammals. J. Mammal. 78, 274–293.

Hiendleder, S., Mainz, K., Plante, Y., Lewalski, H., 1998. Analysis of mitochondrial DNA indicates that domestic sheep are derived from two different ancestral sources: no evidence for contributions from urial and argali sheep. J. Hered. 89, 113–120.

Hollowell, J.G., Staehling, N.W., Flanders, W.D., et al., 2002. Serum TSH, T4 and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J. Clin. Endocrinol. Metab. 87, 489–499.

Horrobin, D.F., 2001. The Madness of Adam and Eve: How Schizophrenia Shaped Humanity. Bantam Press, London.

Horrobin, D.F., 1997. Fatty acids, phospholipids, and schizophrenia. In: Yehuda, S., Mostofsky, D.I. (Eds.), Handbook of Fatty Acid Biology. Humana Press, Totowa, NJ, pp. 245–256.

Horrobin, D.F., Bennett, C.N., 1999. Depression and bipolar disorder: relationships to impaired fatty acid and phospholipids metabolism and to diabetes, cardiovascular disease, immunological abnormalities, cancer, aging and osteoporosis: possible candidate genes. Prostagl. Leukotr. Essent. Fatty Acids 60, 111–167.

Jansen, T., Forster, P., Levine, M.A., Oelke, H., Hurles, M., Renfrew, C., Weber, J., Olek, K., 2002. Mitochondrial DNA and the origins of the domestic horse. Proc. Natl. Acad. Sci. USA 99, 10905–10910.

Jenkins, K., 2001. Not tonight, dear—I'm feeling better: the drugs that relieve depression also sap the libido. McClean's 114 (46), 40–42.

Kaptein, E.M., Hays, M.T., Ferguson, D.C., 1994. Thyroid hormone metabolism: a comparative evaluation. In: Ferguson, D.C. (Ed.), Thyroid Disorders, Veterinary Clinics of North America Small Animal Practice, vol. 24. W.B. Saunders Co, Philadelphia, pp. 431–463.

Kelly, J., 2002. Life-history evolution in Miocene and extant apes. In: Minugh-Purvis, N., McNamara, K. (Eds.), Human Evolution through Developmental Change. Johns Hopkins University Press, Baltimore, pp. 223–248.

Kitajka, K., Puskas, L.G., Zvara, A., et al., 2002. The role of n-3 polyunsaturated fatty acids in brain: modulation of rat brain gene expression by dietary n-3 fatty acids. Proc. Natl. Acad. Sci. USA 99, 2619–2624.

Koop, B.F., Burbidge, M., Byun, A., Rink, U., Crockford, S.J., 2000. Ancient DNA evidence of a separate origin for North American indigenous dogs. In: Crockford, S.J. (Ed.), Dogs Through Time: An Archaeological Perspective, British Archaeological Reports S889. Oxford, pp. 271–286.

Korf, H-W., 1994. The pineal organ as a component of the biological clock: phylogenetic and ontogenetic considerations, In: Pierpaoli, W., Regelson, W., Fabris, N. (Eds.), The Aging Clock: The Pineal Gland and Other Pacemakers in the Progression of Aging and Carcinogenesis. Ann. NY Acad. Sci. 719, 13–42.

Koyama, E., Wu, C., Shimo, T., et al., 2001. Development of stratum intermedium and its role as a sonic hedgehog-signalling structure during odontogenesis. Dev. Dyn. 222, 178–191.

Krings, M., Stone, A., Schmitz, R.W., Krainitzki, H., Stoneking, M., Pääbo, S., 1997. Neanderthal DNA sequences and the origin of modern humans. Cell 90, 19–30.

Krings, M., Capelli, C., Tschentscher, F., et al., 2000. A view of Neanderthal genetic diversity. Nat. Genet. 26, 144–146.

Krumlauf, R., 1994. *Hox* genes in vertebrate development. Cell 78, 191–201.

Kuhn, T.S., 1970. The Structure of Scientific Revolutions. 2nd ed. University of Chicago Press, Chicago.

Lapseritis, J.M., Hayssen, V., 2001. Thyroxine levels in agouti and non-agouti deer mice (*Peromyscus maniculatus*). Comp. Biochem. Physiol. A 130, 295–299.

Lawrence, P.A., Morata, G., 1994. Homeobox genes: their function in *Drosophila* segmentation and pattern formation. Cell 78, 181–189.

Leakey, M.G., Spoor, F., Brown, F.H., et al., 2001. New hominid genus from eastern Africa shows diverse middle Pliocene lineages. Nature 410, 433–440.

Levin, D.A., 2002. Hybridization and extinction. Am. Sci. 90, 254–261.

Lieberman, D.E., 2001. Another face in our family tree. Nature 410, 419–420.

Lightman, S.L., Windle, R.J., Julian, M.D., et al., 2000. Significance of pulsatility in the HPA axis. In: Chadwick, D.J., Goode, J.A. (Eds.), Mechanisms and Biological Significance of Pulsatile Hormone Secretion. J. Wiley and Sons, Chichester, pp. 244–260.

Lindahl, T., 1997. Facts and artifacts of ancient DNA. Cell 90, 1–3.

MacHugh, D.E., Bradley, D.G., 2001. Livestock genetic origins: goats buck the trend. Proc. Natl. Acad. Sci. USA 98, 5382–5384.

Luikart, G., Gielly, L., Exoffier, L, Vigne, J.-D., Bouvet, J., Taberiet, P., 2001. Multiple maternal origins and weak phylogeographic structure in domestic goats. Proc. Natl. Acad. Sci. USA 98, 5927–5932.

McNabb, A.F.M., Wilson, C.M., 1997. Thyroid hormone deposition in avian eggs and effects on embryonic development. Am. Zool. 37, 553–560.

McKinney, M.L., 1998. The juvenilized ape myth—our 'overdeveloped' brain. BioScience 48, 109–116.

McKinney, M.L., 2002. Brain evolution by stretching the global mitotic clock of development. In: Minugh-Purvis, N., McNamara, K. (Eds.), Human Evolution through Developmental Change. Johns Hopkins University Press, Baltimore, pp. 173–188.

McNamara, K.J., 1995. Evolutionary Change and Heterochrony. J. Wiley & Sons, Chichester.

Mannen, H., Tsuji, S., Loftus, R.T., Bradley, D.G., 1998. Mitochondrial DNA variation and evolution of Japanese black cattle (*Bos taurus*). Genetics 150, 1169–1175.

Manzi, G., Gracia, A., Arsuaga, J.-L., 2000. Cranial discrete traits in the Middle Pleistocene humans from Sima de los Huesos (Sierra de Atapuerca, Spain): does hypostosis represent any increase in 'ontogenetic stress' along the Neanderthal lineage? J. Hum. Evol. 38, 425–446.

Martin, B.C., Warram, J.H., Krolewski, A.S., Bergman, R.N., Soeldner, J.S., Kahn, C.R., 1992. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. Lancet 340, 925–929.

Mayr, E., 1982. The Growth of Biological Thought: Diversity, Evolution, and Inheritance. Belknap Press of Harvard University, Cambridge, USA.

Mayr, E., 1988. Towards a New Philosophy of Biology: Observations of an Evolutionist. Harvard University Press, Cambridge Mass.

Mayr, E., 1991. One Long Argument: Charles Darwin and the Genesis of Modern Evolutionary Thought. Havard University Press, Cambridge, Mass.

Mellars, P., 1996. The Neanderthal Legacy: An Archaeological Perspective from Western Europe. Princeton University Press, Princeton, N.J.

Minugh-Purvis, N., 1996. The modern human origins controversy: 1984–1994. Evol. Anthropol. 4, 140–146.

Minugh-Purvis, N., 2002. Heterochronic change in the neurocranium and the emergence of modern humans. In: Minugh-Purvis, N., McNamara, K. (Eds.), Human Evolution Through Developmental Change. Johns Hopkins University Press, Baltimore, pp. 479–498.

Noren, J.G., Alm, J., 1983. Congenital hypothyroidism and changes in the enamel of deciduous teeth. Acta Paediatr. Scand. 72, 485–489.

Nunez, E.A., Becker, D.V., Furth, E.D., Belshaw, B.E., Scott, J.P., 1970. Breed differences and similarities in thyroid function in purebred dogs. Am. J. Physiol. 218, 1337–1341.

O'Reilly, D.S., 2000. Thyroid function tests—time for a reassessment. Br. Med. J. 320, 1332–1334.

Othman, S., Philips, D.I.W., Parkes, A.B., Richards Jr, C., Harris, B., Fung, H., 1990. A long-term follow up of postpartum thyroiditis. Clin. Endocrinol. 32, 559–564.

Ovchinnikov, I.V., Götherstö, A., Romanova, G.P., Kharitonov, V.M., Goodwin, W., 2000. Molecular analysis of Neanderthal DNA from the Caucasus. Nature 404, 490–493.

Park, M.A., 1999. Biological Anthropology. 2nd ed.. Mayfield Publishing Co, Mountain View, CA.

Parker, S.T., 2002. Evolutionary relationships between molar eruption and cognitive development in anthropoid apes. In: Minugh-Purvis, N., McNamara, K. (Eds.), Human Evolution through Developmental Change. Johns Hopkins University Press, Baltimore, pp. 305–316.

Ponce de León, M., Zollilofer, C.P.E., 2001. Neanderthal cranial ontogeny and its implications for late hominid diversity. Nature 412, 534–538.

Porterfield, S.P., 1994. Vulnerability of the developing brain to thyroid abnormalities; environmental insults to the thyroid system. Environ. Health Perspect. 102 (Suppl. 2), 125–130.

Ramirez Rozzi, F., 2002. Enamel microstructure in hominids. In: Minugh-Purvis, N., McNamara, K. (Eds.), Human Evolution through Developmental Change. Johns Hopkins University Press, Baltimore, pp. 319–348.

Reed, K.E., 1997. Early hominid evolution and ecological change through the African Plio-Pleistocene. J. Hum. Evol. 32, 289–322.

Refetoff, S., Weiss, R.E., Usak, S.J., 1993. The syndromes of resistance to thyroid hormones. Endocrinol. Rev. 14, 348–399.

Relethford, J.H., 1997. The Human Species. Mayfield Publishing Co, Mountain View, CA.

Relethford, J.H., 2001. Ancient DNA and the origin of modern humans. Proc. Natl. Acad. Sci. USA 98, 390–391.

Reppert, S.M., Weaver, D.R., 2001. Molecular analysis of mammalian circadian rhythms. Annu. Rev. Physiol. 63, 647–676.

Reppert, S.M., Weaver, D.R., 2002. Coordination of circadian timing in mammals. Nature 418, 935–941.

Risinger, R.K., Proffit, W.R., 1997. Continuous overnight observation of human premolar eruption. Arch. Oral Biol. 41, 779–789.

Schaefer, F., 2000. Pulsatile parathyroid hormone secretion in health and disease. In: Chadwick, D.J., Goode, J.A. (Eds.), Mechanisms and Biological Significance of Pulsatile Hormone Secretion. J. Wiley and Sons, Chichester, pp. 224–243.

Schew, W.A., McNabb, F.M.A., Scanes, C.G., 1996. Comparison of the ontogenesis of thyroid hormones, growth hormone, and insulin-like growth factor-I in ad libitum and food-restricted (altricial) European starlings and (precocial) Japanese quail. Gen. Comp. Endocrinol. 101, 304–316.

Schillaci, M.A., Froehlich, J.W., 2001. Non-human primate hybridization and the taxonomic status of Neanderthals. Am. J. Phys. Anthropol. 115, 157–166.

Schreiber, G., Richardson, S.J., Prapunpoj, P., 2001. Structure and expression of the transthyretin gene in the choroids plexus: a model for the study of the mechanism of evolution. Microsc. Res. Tech. 52, 21–30.

Schreibman, M.P., Scanes, C.G., Pang, P.K.T., 1993. The Endocrinology of Growth, Development and Metabolism of Vertebrates. Academic Press, New York.

Schoeninger, M., 1982. Diet and evolution of modern human form in the Middle East. Am. J. Phys. Anthropol. 58, 21–53.

Schwartz, G.T., Dean, C., 2001. Ontogeny of canine dimorphism in extant hominoids. Am. J. Phys. Anthropol. 115, 269–283.

Schwartz, J.H., 1999. Sudden Origins: Fossils, Genes, and the Emergence of Species. J. Wiley and Sons, New York.

Shea, B.T., 1992. A developmental perspective on size change and allometry in evolution. Evol. Anthropol. 1, 125–134.

Short, R.V., Balaban, E., 1994. The Differences Between the Sexes. Cambridge University Press, Cambridge, UK.

Speake, B.K., Surai, P.F., Bortolotti, G.R., 2002. Fatty acid profits of yolk lipids of five species of wild ducks (Anatidae) differing in dietary preferences. J. Zool. Lond. 257, 533–538.

Sponheimer, M., Lee-Thorpe, J.A., 1999. Isotopic evidence for the diet of an early hominid, *Australopithecus africanus*. Science 283, 368–369.

Stringer, C., 2002. Modern human origins: progress and prospects. Philos. Trans. R. Soc. Lond. B 357, 563–579.

Stringer, C., Gamble, C., 1993. In Search of the Neanderthals: Solving the Puzzle of Human Origins. Thames and Hudson, New York.

Susman, R.L., 1994. Fossil evidence for early hominid tool use. Science 265, 1570–1573.

Talbot, S.L., Shields, G.F., 1996. A phylogeny of the bears (Ursidae) inferred from complete sequences of three mitochondrial genes. Mol. Phylogenet. Evol. 5, 567–575.

Tattersall, I., Schwartz, J.H., 2001. Extinct Humans. Westview Press, Boulder.

Trinkaus, E., Shipman, P., 1993. The Neanderthals: Changing the Image of Mankind. Knopf, New York.

Trueman, J.W.H., 2001. Does the Lake Mungo 3 mtDNA evidence stand up to analysis? Arch. Oceania 36, 163–165.

Trut, L.N., 1999. Early canid domestication: the farm-fox experiment. Am. Sci. 87, 160–169.

Vanderpump, M.P.J., Turnbridge, W.M.G., French, J.M., Appleton, D., Bates, M., Clark, F., 1995. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham survey. Clin. Endocrinol. 43, 55–68.

Velduis, J.D., 2000. Nature of altered pulsatile hormone release and neuroendocrine network signalling in human ageing: clinical studies of the somatotropic, gonadotropic, corticotropic and insulin axes. In: Chadwick, D.J., Goode, J.A. (Eds.), Mechanisms and Biological Significance of Pulsatile Hormone Secretion. J. Wiley and Sons, Chichester, pp. 163–189.

Voss, S.R., Shaffer, H.B., 1997. Adaptive evolution via a major gene effect: paedomorphosis in the Mexican axolotl. Proc. Natl. Acad. Sci. USA 94, 14185–14189.

Ward, R., Stringer, C., 1997. A molecular handle on the Neanderthals. Science 388, 225–226.

Waterman, A.J., 1958. Development of the thyroid–pituitary system in warm-blooded amniotes. In: Gorbman, A. (Ed.), Comparative Endocrinology. John Wiley & Sons, New York, pp. 351–367.

Weetman, A.P., 1997. Hypothyroidism: screening and subclinical disease. Br. Med. J. 314, 1175–1178.

Weissman, M.M., 2001. Treatment of Depression: Bridging the 21st Century. American Psychiatric Press Inc, Washington, DC.

Windle, R.J., Wood, S.A., Lightman, S.L., Ingram, C.D., 1998. The pulsatile characteristics of hypothalmo–pituitary–adrenal activity in female Lewis and Fischer 344 rats and its relationship to differential stress responses. Endocrinology 139, 4044–4052.

Williams, F.L., Godfrey, L.R., Sutherland, M.R., 2002. Heterochrony and the evolution of Neanderthal and modern craniofacial form. In: Minugh-Purvis, N., McNamara, K. (Eds.), Human Evolution through Developmental Change. Johns Hopkins University Press, Baltimore, pp. 405–441.

Wolpoff, M.H., 1999. Paleoanthropology. 2nd ed. McGraw-Hill, Boston.

Wolpoff, M.H., Hawks, J., Frayer, D.W., Hunley, K., 2001. Modern human ancestry at the peripheries: a test of the replacement theory. Science 291, 293–297.

Wright, M.L., 2002. Melatonin, diet rhythms, and metamophosis in anuran amphibians. Gen. Comp. Endocrinol. 126, 251–254.

Yeh, J., 2002. The effect of minaturized body size on skeletal morphology in frogs. Evolution 65, 628–641.